BIOLOGICAL CONTROL OF WEEDS LABORATORY - EUROPE

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BIOLOGICAL CONTROL OF WEEDS LAB PERSONNEL

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Cover Photograph

Cheilosia corydon (Harris) (=*grossa* Fallen)
Diptera: Syrphidae

The larvae of this fly seriously damage the stems of musk thistle. This is an important candidate for the biological control of that weed.

Photo by Pasquale Pecora and Massimo Stazi

NOT FOR PUBLICATION

NOTICE

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Introduction

The year 1984 was an important year for the Rome laboratory, because as a result of the 1983 laboratory review we were able to concentrate our research efforts on a few important weeds and the results are reflected by the in-depth reports of most of the investigators.

Among the major accomplishments was the petitioning for the release of Bangasternus orientalis on yellow starthistle. The data for this petition were the result of the closely combined efforts of the researchers at the Rome, Albany, and Thessaloniki laboratories. Also initial trials were finished on Aphthona abdominalis, (flea beetle) and the seed gall midge, Dasineura capsulae, both candidates for leafy spurge control.

In addition to the finishing of the work on B. orientalis other important, basic work on yellow starthistle that needed to be done has been started. Specifically, the taxonomic study of the Apion spp. and trypetid fly complex associated with the plant, and studies on the host utilization patterns of the seed head insects were undertaken.

Another valuable contribution was the discovery of a cold hardy population of the leafy spurge tip-gall midge (Bayeria capitigena) in Austria. Representatives of this population have been sent to Albany for final testing. Our work on leafy spurge also moved ahead with the discovery of a biotype of Chamaesphecia sp. infesting Euphorbia virgata. This may be the biotype that will accept and damage leafy

spurge in N. America. On the same survey a colony of *Simyra*, probably *dentinosa*, whose larvae completely defoliate spurge plants, was found on *E. virgata*. This discovery will be exploited in the future.

The work on knapweeds saw the final host specificity testing at Rome of *Pterolonche inspersa* whose larvae mine the roots of diffuse knapweed and the beginning of work with another candidate, the weevil *Bangasternus provincialis*.

Work on musk thistle provided us with a good understanding of the adult biology of *Cheilosia corydon* a phytophagous syrphid fly that ravages *Carduus* spp. It is this fly that is figured on the front cover of this report.

We at the Rome laboratory are proud but not content with our accomplishments this year. Next year should be even more productive.

Paul H. Dunn

Paul H. Dunn

Research Leader

EUPHORBIA ESULA VIRGATA "COMPLEX" (Leafy spurge)

P. Pecora and M. Cristofaro

1) Dasineura capsulae (Diptera: Cecidomyiidae)

The results of preliminary testing conducted in the 1983 demonstrated that under laboratory conditions the gall midge Dasineura capsulae was able to oviposit and develop on some North American biotypes of leafy spurge (i.e. Euphorbia esula-virgata "complex") from Montana and Oregon. These indications led us to conduct more extensive testing, to determine the host range of this midge. To that end both laboratory and field trials were carried out and additional bionomical observations were made in 1984. Lastly, data on the blooming period of leafy spurge were also obtained.

Field experiment

Multiple Choice Host Suitability Test - The object of this experiment was to determine if, in a field situation, feral adults of D. capsulae were able to select any of the test-plants as hosts. The experiment was conducted at S. Rossore (Pisa) Italy, where a population of this midge and its host (E. esula) occur naturally.

The experimental area was selected in June 1983 because about 50% of the E. esula plants, which occurred in dense stands, were infested by D. capsulae larvae. The area, about 10 x 50 m was situated along a river canal and was left undisturbed until April 16 1984 when the experiment was started. . The following test-plants were included in the trial: Euphorbia esula-virgata "complex" from Nebraska, Montana, Wyoming and Oregon; Euphorbia peplus, E. milii, E. characias, E.

heterophylla, E. pulcherrima, Rosa sp., Thymus serpyllum, Ruta graveolens, Nerium oleander, Lilium sp., Linum usatissimum, Rosa sp., Clarkia elegans, Pelargonium zonale and Medicago sativa.

The test-plants were transplanted in 22 cm. plastic pots and two different controls were also included. One control was E. esula plants from S. Rossore transplanted into the same size pots as the test plants (control A); and Control B ie. E. esula plants growing naturally in the experimental area.

The period of keeping the plants in pot before use in the experiment, was not uniform. The plants of leafy spurge from Montana, Nebraska and Wyoming were kept in pots for six months prior to the experiment while the plants from Oregon were in pots for 18 months and the plants of Control A were in pots for 12 months prior to use. For the other test plants this period ranged between four and six months.

Within the experimental area there were six square (4.00 m x 4.00 m) plots. Each plot, divided into 25 squares (0.80 m x 0.80 m/square), was treated as follows:

a) The plastic pots containing the test plants, were placed in the ground with the top at ground level.

b) Each square received a test plant or a control plant.

c) Each plot received three test plants, plus the control A and control B.

d) Each test plant and the controls (A and B) had five replications in the same plot.

e) The test plants and the controls were located randomly in the plots.

The naturally growing plants of leafy spurge, except Control B, were removed from the test plot leaving only the test plants plus the controls.

In order to follow infestation and gall development, weekly inspections were conducted from mid-April to the end of May. For each test plant species, the number of plants present/pot, plus the number of buds, flowers and galls/plant were recorded.

Results

The data obtained in this field experiment clearly indicate that *D. capsulae* adults were able to select as hosts only plants of the *Euphorbia esula-virgata* group. The results of this experiment are summarized in Table 1.

The North American biotypes of leafy spurge from Montana, Oregon and Wyoming were shown to be suitable hosts, but no galls were observed on the Nebraska biotype. Both controls (A and B) were also attacked by *D. capsulae*, but no galls were formed on the following test plants: *Euphorbia peplus*, *E. milii*, *Pelargonium zonale*, *Nerium oleander*, *Thymus serpyllum* and *Ruta graveolens*. *Euphorbia characias* and *Poinsettia pulcherrima* were not in the right stage during the emergence period of the midge and the other test plants (*Lilium* sp., *Linum usatissimum*, *Rosa* sp., *Euphorbia heterophylla*, *Clarkia elegans* and *Medicago sativa*), were damaged by animals, so no valid results were produced by these test plants.

It was also noticed that significantly more galls were obtained on the control plants growing naturally (Control B) than on the Control plants in pots (Control A).

During the various inspections, from mid-April to the end of May, it was observed that potted plants of leafy spurge had some chlorotic basal leaves and about 50% of the flower buds fell before reaching maturity, while the leaves and buds of control B (the naturally growing plants) were in good vegetative condition during this period.

The poor condition of the plants in control A may have been due to a lack of nutrients because, these plants had been kept in pots for a year without fertilization.

The lower infestation of *D. capsulae* recorded on the potted plants of the control A, could be because the oviposition of this midge occurred at the time when the number of suitable flower buds was drastically reduced, therefore there was less possibility for the ovipositing *D. capsulae* to select the inflorescences in the appropriate stage.

Another possible explanation is that oviposition of *D. capsulae* occurred when a large number of suitable flower-buds was still available, but about half of the flower buds dropped before reaching maturity because of the plant's weakened condition.

Laboratory Experiments

In order to determine the host range of *D. capsulae* under forced conditions, two experiments (No choice oviposition and host suitability test; No choice host suitability test) were conducted in the laboratory, using three different populations of *D. capsulae*: one from S. Rossore (Pisa) Italy collected on *E. esula*, another one from Austria collected on *E. virgata*, and a third population collected from *E. virgata* in Hungary.

No choice oviposition and host suitability test

To provide vigorous adults of *D. capsulae* to use in the laboratory trial, 300 bud galls containing midge larvae of various stages were collected on *E. esula* at S. Rossore (Pisa) Italy, on June 11, 1983.

These galls were brought to the laboratory and stored in a

refrigerator in a closed polyethylene bag (temp. 4^{o}-6^{o}C) for a period of 4-7 days. The low temperature and high humidity allowed to the mature larvae of D. capsulae to leave the galls. When the larvae started to come out, they were transferred, using a fine brush, into plastic boxes (L = 10 cm; W = 10 cm; H = 16 cm) with a layer (2 cm) of moistered peat moss on the bottom to provide a suitable substrate for the diapausing insects. Each box was covered by a plastic lid which had a 2 cm diameter central hole plugged with cotton to allow some air exchange. Ten containers were prepared (200 mature larvae/container) and held in an outdoor insectary. These containers were checked periodically until adult emergence was noticed, then they were checked daily.

Newly emerged adults were transferred, by a mouth aspirator, into acrylic plastic cages (H = 10cm; diam 6 cm) covered by nylon organdy. A 1.5 cm diameter hole was made through the wooden cage bottom through which the flower buds of the test plants were passed, thus allowing the insects to be caged directly on the plant. These cages were supported on the plants by fastening them with masking tape to metal rod inserted in the soil of the potted plant. A range of 8-25 flower buds/cage were exposed to 4-6 adults of D. capsulae and 2-3 replications were made for each test plant. . The cages containing the adult midges were held in a laboratory room, where the ambient temperature ranged between 21^{o}C 25^{o}C and were left undisturbed until the midges died. Later, in order to determine if oviposition had occurred, we dissected the flower buds until eggs of D. capsulae were found in 1 or 2 of them. Once we had ascertained that oviposition had occurred, the other flower buds were left undisturbed to follow the gall development and these plants were transferred outside to the laboratory garden, but kept in their pots.

To determine the percent of egg hatch, the eggs found in the bud dissections were placed in a 35 cc plastic cup which had a layer (2 cm thick) of moist plaster of Paris on the bottom and was closed by a plastic cap.

The following test-plants were tested with the insects of Italian origin: E. esula-virgata "complex" from Montana, Nebraska, and Wyoming, Pelargonium zonale plus the control (E. esula from S. Rossore).

The Austrian population, (100 bud galls) was collected on June 15, 1983 near Alland (Vienna area) on E. virgata. This material was manipulated as explained above. Three containers were prepared (80 larvae/container). E. esula-virgata "complex" from Montana and E. peplus plus the control were tested with this population.

The Hungarian population of D. capsulae (90 bud galls) was collected on E. virgata on June 10, 1983 in the Debrecen area. Two containers (80 mature larvae/container) were prepared. E. esula-virgata "complex" from Montana and E. peplus were exposed to the population of this midge, but no control plants from Hungary were available. The experiment started on April 22 and ended on June 5, 1984.

Results

The data obtained in this laboratory experiment demonstrated that the Italian adults of D. capsulae were able to oviposit, in captivity, on different biotypes of North American leafy spurge (E. esula-virgata "complex" from Montana, Nebraska, and Wyoming). Moreover oviposition occurred on E. esula-virgata "complex" from Austria as well as on the control (E. esula from S. Rossore, Italy). No eggs were found on Pelargonium zonale. The eggs of D. capsulae, collected from bud

dissections of the exposed leafy spurges, were fertile. The percent of egg hatch varied from 45.5% (eggs layed on E. esula-virgata "complex" Wyoming type) to 91.5% (eggs layed on E. esula-virgata "complex" Montana type). Gall development occurred on the test plants where oviposition occurred, except for Nebraska type. On this test plant, six flower buds were examined to ascertain if oviposition occurred and a total of 107 D. capsulae eggs were counted in three of them and 71.9% of these eggs were fertile.

The lack of development of D. capsulae galls on E. esula-virgata "complex" Nebraska type may have been due to the fact that these plants kept in pots for six months had a nutrient deficiency thus lacking vigor the buds did not support gall formation. This negative result could also be due to the lack of suitability of this taxon, which precludes the larval development of this midge.

The population of Dasineura from Austria oviposited and galls developed on E. esula-virgata "complex" Montana type, as well as on the Austrian control. Oviposition did not occur on E. peplus.
The insects from Hungary also oviposited on the Montana plants and no eggs were found on E. peplus. All these results are shown in Table 2.

No Choice Host Suitability Test

Adults of D. capsulae, from Italy, Austria and Hungary, once emerged were placed into acrylic plastic cages as described above. The potted test plants with the caged insects were kept in a laboratory room where the ambient temperature ranged between 20°C and 25°C, until the insects died. The cages were then removed, the plants which had been exposed to the midges were moved to a shaded area in the laboratory garden and left undisturbed, but kept under observation for the formation of Dasineura induced galls.

The Italian population was exposed to the following test plants: E. esula-virgata "complex" from Montana, Nebraska and Wyoming, E. milii, E. peplus, Rosa sp. and the E. esula control from San Rossore.
The insects from Austria were tested with E. esula-virgata "complex" from Montana and Wyoming, plus the E. virgata control from Alland, Austria.
E. esula-virgata "complex" from Montana and E. peplus were tested with the Hungarian population of this midge, but no control was included, because plants from Hungary were not available. The trial was conducted in the same period of the previous experiment.

Results

Italian biotype Dasineura:. Substantial gall development occurred on North American leafy spurge (E. esula-virgata "complex" from Montana and Wyoming) and on the E. esula control from San Rossore, Italy). Two galls were found on the leafy spurge from Nebraska. No galls were found on E. peplus and Rosa sp.

The Austrian biotype Dasineura accepted the biotypes of North American leafy spurge from Montana and Wyoming as hosts and gall development also occurred on the control from Austria.
Leafy spurge from Montana was a suitable host for the D. capsulae coming from Hungary, but no galls developed on E. esula-virgata from Nebraska. For this test, no control from Hungary was available.
The results of this trial are shown in Table 3.

Bionomical observations

The larvae of D. capsulae overwinter in the soil. In the laboratory it was observed that the overwintering larvae move towards

the soil surface, where they pupate. Once the midge pupates, the new adult will emerge in 1-2 days.

D. capsulae usually lays eggs in groups in the inner part of the bracts of the flower bud of E. esula, but sometimes the oviposition was also observed directly into the cyathium.

The adults of the Italian population (ex. E. esula) collected as larvae in 1983 started to emerge in the laboratory on April 6, 1984. From 2000 mature larvae, kept in plastic pupation cages, 176 adults (8.8%) emerged. The sex ratio was 96 ♀ and 80 ♂. In addition, 56 adult parasitoids of the genera Inostemma and Pseudotorymus also emerged.

The Austrian population of this midge started to emerge on April 6, 1984 and the emergence continued until May 4. From 240 mature larvae, 50 adults (30 ♀ 20 ♂) emerged (20.8%). Twelve parasitoids were also collected but have not yet been identified.

From 160 mature larvae collected in Hungary, 34 adults (22 ♀ 12 ♂) emerged (21.2%). The emergence period lasted from April 24 to May 4, 1984. Ten parasites were also colleced but are not yet identified.

Observations on the Phenology of Leafy Spurge

In order to better understand the life cycle of D. capsulae at San Rossore it was necessary to have information on the the budding and blooming period of E. esula, so periodic observations were made at irregular intervals at San Rossore from April to June, 1984.

On April 3, 1984, in an area, where a natural infestation of leafy spurge occurred, two sites were selected: Site A was a dense stand of about 300 mq in an open area while Site B was a dense stand of about 200 mq in a half-shaded area.

For each site 30 randomly selected young plants of E. esula were marked by metal rods inserted in the soil near them and were inspected on April 3, 11 and 18; May 11, 18 and 31; June 7 and 19.

For the first three inspections, when the plants had not yet reached the bud stage, only the height of the marked plants was measured. Later, when the plants had reached the flower bud stage, for each inspection five plants were collected at random, and the height, the number of buds, flowers and fruits/plant were measured and recorded.

Results

In the sites A and B, some plants of E. esula marked on April 3 had reached the bud stage by April 11 (21 plants in site A and 16 in site B). The inflorescences of the first plant sampling made on May 4 had only buds (61.50 ± 1.38 buds/plant - site A - and 39.27 ± 14.34 buds/plant - site B -). In the May 18 sample, the plants contained both buds and flowers (9.80 ± 10.11 buds/plant and 72.40 ± 17.98 flowers/plant - site A; 39.40 ± 41.16 buds/plant and 74.00 ± 37.70 flowers/plant - site B) and on May 31 flowers and fruits were found, but no buds (57.8) ± 48.30 flowers/plant and 22.00 ± 11.85 fruits/plant - site A -; 42.80 ± 39.24 flowers/plant and 75.80 ± 34.77 fruits/plant - site B -). On June 7 there were no buds, only older flowers and fruits continued to be found (32.20 ± 22.52 flowers/plant and 32.20 ± 32.08 fruits/plant) and on June 19 the flowers were all finished and only fruits were found (75.00 ± 8.66 fruits/plant - site A -; 116.00 ± 72.83 fruits/plant - site B -).

The 86% and 100% of the E. esula plants of the site A and B which were examined early in the season produced buds and flowers.

These data confirmed the syncronization between the emergence

period of D. capsulae and an abundance of flower buds of its host plant. Based on observations conducted in 1983, we assumed that the majority of adults of D. capsulae would emerge at the time when the E. esula plants are in bud stage. In fact, about 90% of the galls produced by the developing larvae of this midge at San Rossore started by the enlargement of the developing cyathium, while it was still protected by bracts.

Table No. 1. Results of the multiple choice host suitability test conducted at San Rossore (Pisa) Italy 1984 1/.

PLANTS	No.of plants	No. of plants/rep $\bar{x} \pm SD$	No. of infested plants	No. of infested replicates	No. of flowers/plant $\bar{x} \pm SD$	No. of galls/plant $\bar{x} \pm SD$	% of flowers with galls $\bar{x} \pm SD$
			PLOT 1				
CONTROL A 1/	13	2.6 ± 1.82	7	4	26.22 ±14.92	3.33 ± 2.36	12.53 ± 12.90
CONTROL B 1/	40	8 ± 5.38	38	4	69.4 ± 50.87	11.47 ± 9.71	15.00 ± 10.10
Leafy spurge ex Nebraska	6	1.2 ± 0.45	0	0	10.33 ± 13/41	0	0
Medicago sativa L. 2/							
Pelargonium zonale (L.) 2/							
			PLOT 2				
CONTROL A 1/	9	1.8 ± 1.3	1	1	32.78 ± 24.83	0.56 ± 1.67	1.11 ± 3.33
CONTROL B 1/	30	6.0 ± 1.73	27	5	69.64 ± 45.44	13.00 ± 7.92	18.84 ± 11.28
Leafy spurge ex Oregon	9	1.8 ± 0.84	3	2	30.44 ± 13.77	4.44 ± 9.25	8.28 ± 13.27
Leafy spurge ex Wyoming	6	1.2 ± 0.45	2	2	24.67 ± 23.21	2.33 ± 3.61	3.33 ± 4.75
Euphorbia heterophylla 2/ Linn, Amoeana							
			PLOT 3				
CONTROL A 1/	12	2.4 ± 1.67	6	2	19.17 ± 17.94	3.17 ± 5.29	8.15 ± 12.73
CONTROL B 1/	16	3.2 ± 1.20	11	5	54.69 ±40.10	10.06 ± 9.63	13.64 ± 12.51
Leafy spurge ex Montana	10	2 ± 1.00	5	3	33.80 ±28.67	2.10 ± 3.51	7.80 ± 11.90
Euphorbia milii Ch. des Moulins	5	1	0	0	4 ± 1.58	0	0
Lilium sp. cv. Tabasco 2/							

PLOT 4

CONTROL A[1/]	9	1.8 ± 1.09	4	3	14.44 ± 17.93	7.78 ± 14.33	18.12 ± 25.18
CONTROL B[1/]	12	2.4 ± 0.55	11	5	40.67 ± 26.59	13.92 ± 8.84	30.66 ± 27.67
Euphorbia pulcherrima Willd. ex. Kl.	5	1	0	0	0	0	0
Nerium oleander L.	5	1	0	0	15.2 ± 6.10	0	0
Thymus serpyllum L.	5	1	0	0	329.2 ± 36.79	0	0

PLOT 5

CONTROL A[1/]	11	2.2 ± 1.30	4	3	18.64 ± 18.99	3.91 ± 6.59	7.85 ± 11.53
CONTROL B[1/]	19	3.8 ± 1.64	18	5	55.56 ± 43.68	15.44 ± 9.18	28.82 ± 21.19
Euphorbia peplus L.	10	2	0	0	230.6 ± 65.51	0	0
Rosa sp.[2/]							
Linum narbonense L.[2/]							

PLOT 6

CONTROL A[1/]	8	1.6 ± 0.89	3	2	13.12 ± 14.86	1.75 ± 2.66	17.21 + 34.72
CONTROL B[1/]	20	4 ± 2.34	16	5	63.44 ± 51.81	19.11 ± 13.28	24.42 ± 17.20
Euphorbia characias L.	10	2	0	0	0	0	0
Clarkia elegans Dougl.							
Ruta graveolens L.	5	1					

1/ *Euphorbia esula* San Rossore, ex Pisa.

2/ Plants either damaged by animals or not in the "right stage".

Table No. 2. No Choice Oviposition and Host Suitability Test

PLANTS	No. of replicates	Total No.of Buds exposed	No.of dissected buds (No.of buds with eggs)	Oviposition (No. eggs)	% of hatching	No. of buds left for gall development	No. of Galls
Dasineura ex Euphorbia esula - San Rossore (Italy) type							
Control							
Euphorbia esula ex Pisa	1	24	8 (2)	40	87.50	16	11
Euphorbia esula-virgata ex Nebraska	2	43	13 (3)	107	71.96	36	-
Euphorbia esula-virgata ex Wyoming	2	32	11 (1)	11	45.45	32	23
Euphorbia esula-virgata ex Montana	3	57	19 (4)	95	91.58	38	11
Euphorbia virgata ex Austria	2	40	23 (3)	44	88.64	17	8
Pelargonium zonale L.	2	49	49	-	-	0	-
Dasineura ex Euphorbia virgata - Alland (Austria)							
Euphorbia esula-virgata ex Montana	3	36	25 (4)	87	82.5	11	8
Euphorbia peplus L.	2	58	58 (-)	-			
Euphorbia esula-virgata ex Austria (Control)	2	31	8 (5)	60	78.3	23	15
Dasineura ex Euphorbia virgata - Debrecen (Hungary) type [a/]							
Euphorbia esula-virgata ex Montana	1	10	3 (1)	9	100	7	2
Euphorbia peplus L.	1	7	7 (-)	-			

a/ Control plant from Hungary was not available.

Table No. 3. No Choice Host Suitability Test

LANTS	No. of replicates	Total No. of buds exp.	Total No. of Galls	% Flowers with galls
Dasineura ex Euphorbia esula - San Rossore (Italy) type				
ONTROL				
uphorbia esula ex Pisa	4	105	14	13.33
uphorbia esula-virgata x Montana	4	81	33	40.74
uphorbia esula-virgata x Wyoming	4	49	29	48.98
uphorbia esula-virgata x Nebraska	5	52	2	3.85
uphorbia milii Ch. des Moulins	3	8	0	0
uphorbia peplus L.	3	54	0	0
osa sp.	2	6	0	0
Dasineura ex Euphorbia virgata - Debrecen (Hungary) type				
uphorbia esula-virgata x Nebraska	3	33	0	0
uphorbia esula-virgata x Montana	3	60	11	18.33
Dasineura ex Euphorbia virgata - Alland (Austria) type				
uphorbia esula-virgata x Austria (Control)	2	30	13	43.30
uphorbia esula-virgata x Montana	3	44	12	27.27
uphorbia esula-virgata x Wyoming	2	18	11	61.11

2) Aphthona abdominalis (Coleoptera: Chrysomelidae)

Since the results of the preliminary testing conducted in 1983, demonstrated that adults of Aphthona abdominalis (Duftsch.) fed only on plants in the genus Euphorbia, more detailed tests (adult no choice feeding test, adult multiple choice feeding test) were carried out in 1984, in order to better define the host plant range of this flea beetle.

Adult No Choice Feeding Test

To establish the host-plant range of A. abdominalis, an experiment was conducted at the Rome laboratory, exposing 51 plant species or varieties in 22 families to this insect. Included in this plant list were species in the order Euphorbiales and other orders of the superorder Rosidae, closely related to Euphorbia, economic or ornamental plants in other superorders plus plant species attacked by other Aphthona spp. (Table 1). Heywood's Flowering Plants of the World (1978) was used as a guide in constructing our test list.

For use in the experiment, adults of A. abdominalis were collected on leafy spurge plants from mid-July to the beginning of August, 1984, as they emerged from the soil, at the Rome laboratory garden. This population was established from a colony of A. abdominalis, which came from S. Rossore (Pisa) Italy, in 1982.

Since there were no apparent external morphological characters to separate living males and females, unsexed insects were used in the experiment and the sex of insects was determined after death by dissecting them under a stereomicroscope.

Paper cups (200 cc) with perforated plastic lids (to allow

aeration) were used as cages. Five adults of A. abdominalis were placed in each cup and the test plants were added as bouquets and replaced twice weekly. The feeding damage was estimated by placing a transparent grid divided into 1 mm squares over the leaves which had been damaged and counting the mm^2 of leaf that had been consumed. Also a record was made of any insects which had died since the last bouquet change.

The test started on July 13 and ended on October 22, 1984.The results are reported as mm^2 eaten per insect per day on a test plant and as percent feeding [(mm^2 eaten per insect per day on test plant/mm^2 eaten per insect per day on control) x 100].
This experiment was conducted in a laboratory room, with a natural light, at temperatures ranging between 18°C and 25°C.

Results

Of 51 plant species or varieties in 20 families exposed to adults of A. abdominalis, feeding occurred on 20 test-plants, mainly on species of Euphorbiaceae, eg. species in the subgenus Esula, Chamaesyce, Euphorbium and Agaloma. Besides the Euphorbiaceae, feeding was also noted on plants in the families: Lythraceae, Geraniaceae, Convolvulaceae and Moraceae.

However either the amount of feeding occurring on plants in these families or the insect longevity were significantly reduced when compared with the control plants.

The results of this experiment are summarized in Table 2.

Multiple Choice Feeding Test

To get a more complete picture of the host-range of *A. abdominalis*, a multiple choice feeding test was set up, using some of the plants on which feeding occurred in the previous experiment. Sixteen plants in the Euphorbiaceae, Lythraceae Geraniaceae and Moraceae were selected for this test.

Four plastic boxes (L= 35cm; W= 20cm; H= 18 cm) were used for this test. Each box was modified by replacing the cover with fine mesh screen to provide aeration and by cutting 5 holes in the bottom to hold water-filled vials each containing a bouquet of different plant species. Each box contained four test plants plus the control, without replication. Plant species were randomly assigned and 15 unsexed adults of *A. abdominalis* which had fed previously on *E. esula*, were introduced into each cage. The test was conducted in a laboratory room, where the temperature ranged between 20°C and 26°C, and lasted from July 26 to mid-August. Bouquets were changed twice a week and the feeding damage was estimated as explained in the previous experiment. At the end of the experiment both dead and living adults were dissected to determine the sex.

Results

Feeding occurred on ten of the sixteen plant species included in this test. Plant species that were consumed in large amount in the no-choice feeding test were generally consumed much less when in the presence of leafy spurge. Only on *Ricinus communis* was the percent feeding almost equal to the no choice test, and there was no feeding on *E. antysiphilitica*, *E. characias*, *Codiaeum variegatum*, *Ficus elastica* and *Ipomea*.

Considering feeding damage on the control as 100%, the feeding was: less than 10% on *E. lathyris*, *E. tirucalli* and *Lythrum salicaria*, between 10 and 40% of feeding on the control on *E. peplus*, *E. marginata*, *E. milii* and *Ricinus communis*; between 40 and 60% of control feeding on *E. maculata* and *E. trigona* jumping to 110% of control feeding on *E. lucida*.

Table No. 1 Plants selected for the no-choice feeding test of Aphthona abdominalis.

1) Plants related to leafy spurge (EUPHORBIACEAE)

ORDER	SUBGENUS	SPECIES
Euphorbiales	Esula	* Euphorbia esula L.
		* Euphorbia esula-virgata "complex" Nebraska type
		* Euphorbia esula-virgata "complex" Montana type
		* Euphorbia esula-virgata "complex" Wyoming type
		* Euphorbia lathyris L.
		* Euphorbia characias L.
		* Euphorbia lucida W. et K.
		* Euphorbia peplus L.
	Agaloma	* Euphorbia marginata Pursh.
		* Euphorbia antisyphilitica Zuccar.
	Poinsettia	Euphorbia pulcherrima Willd.
		Euphorbia heterophylla L.
	Euphorbium	* Euphorbia milii Ch. des Moulins
		* Euphorbia tirucalli L.
		* Euphorbia trigona Haw.
		* Euphorbia prob. triangularis Desf.
	Chamaesyche	* Euphorbia maculata L.
		* Euphorbia prostrata Aiton
		* Codiaeum variegatum Blume
		Mercurialis sp.
		* Ricinus communis L.
		Manihot palmata Mull. Arg.

2) Plants attacked by other species of the genus Aphthona

ORDER	FAMILY	SPECIES
Geraniales	Geraniaceae	Geranium rotundifolium L.
		* Pelargonium zonale Ait
	Linaceae	Linum flavum L.
		Linum usitatissimum L.
Myrtales	Lythraceae	* Lythrum salicaria L.
Rosales	Rosaceae	Prunus avium L.
		Rosa sp.
Lamiales	Labiatae	Salvia splendens Ker. Gavl.
		Thymus serpyllum L.
Gentianales	Apocynaceae	Nerium oleander L.
Ebenales	Ebenaceae	Diospyros kaki L.
Juglandales	Juglandaceae	Juglans regia L.
Fabales	Leguminosae	Medicago sativa L.
Sapindales	Rutaceae	Ruta graveolens L.
Rhamnales	Vitaceae	Vitis vinifera L.
Poales	Graminae	Zea mays L.

* = Feeding occurred. For detailed results see Table No. 2

Table 2. Results of the no-choice feeding test of *A. abdominalis*, Rome Lab. 1984.

PLANTS	No.of adults ♀	♂	mm^2 eaten/day per insect $\bar{x} \pm$ SD	Feeding Min-Max (mm^2/day/insect)	% Feeding[1/] $\bar{x}$	Longevity (days) $\bar{x} \pm$ SD
EUPHORBIACEAE						
Euphorbia esula Pisa (control)	1	4	8.15± 3.64	5.71-12.5	100	39.8 ±26.75
E. lucida	2	3	16.52± 5.11	12 -26.25	202.70	28 ± 2.24
E. prob. *triangularis*	2	3	15.5 ±14.42	10.63-41	190.18	31.8 ± 4.6
E. maculata	1	4	14.20±10.93	0 -32.67	174.23	58.2 ±28.43
E. trigona	2	3	9.26± 5.34	4.5 -11.6	113.62	30.12±18.91
E. peplus	1	4	7.87± 1.82	5.93-10.33	96.56	17.6 ± 7.6
E. milii	2	3	7.19± 5.01	0.33-16	88.22	56.2 ± 6.26
E. tirucalli	1	4	6.01± 4.68	0 -14.5	73.74	24.0 ±23.75
E. marginata	2	3	5.92± 6.90	0 -21.67	72.64	46.8 ± 2.19
E. esula-virgata Montana	2	3	4.62± 1.50	2.57- 8.00	56.69	101 ± 0
E. esula-virgata Nebraska	2	3	3.56± 2.15	0 - 6.88	43.68	40.8 ±41.76
E. prostrata	2	3	3.54± 2.28	2.5 - 6.77	43.44	19.4 ±11.33
E. esula-virgata Wyoming	1	3	3.19± 2.12	0.13- 8.00	39.14	76.5 ±28.86
Ricinus communis	2	3	3.07± 3.3	0 - 9.73	37.67	42.6 ±16.39
E. lathyris	3	2	1.25± 2.16	0 - 3.75	15.34	8 ± 3.0
E. antisyphilitica	3	2	1.00± 1.41	0 - 2.00	12.27	7.00± 0.00
Codiaeum variegatum	1	4	0.28± 0.68	0 - 1.67	3.44	10.00± 6.63
E. characias	3	2	0.06± 0.15	0 - 0.33	0.74	8.8 ± 5.89
GERANIACEAE						
Pelargonium zonale	1	4	0.22± 0.39	0 - 0.67	2.70	5.4 ± 3.13
LYTHRACEAE						
Lythrum salicaria	1	4	1.88± 2.06	0 - 4.78	23.07	20.4 ±10.78
MORACEAE						
Ficus elastica	3	2	0.65± 0.94	0 - 2.00	7.98	14 ± 3.08
CONVOLVULACEAE						
Ipomoea alba	2	3	0.11± 0.19	0 - 0.33	1.35	8.6 ± 2.19

1/ % Feeding = $\dfrac{mm^2 \text{ eaten per insect per day on test plant}}{mm^2 \text{ eaten per insect per day on control}} \times 100$

3) Oncochila simplex (Hemiptera: Tingidae) (Pecora, F. Murano - part-time)

The host specificity tests on Oncochila simplex were conducted at the USDA Rome Laboratory in 1979 on 29 species of test plants selected for adult feeding, oviposition and nymphal survival trials. Two of these were thyme (Thymus serpyllum) and tansy ragwort (Senecio jacobaea), 21 were Euphorbia species, and the remainder were species from genera related to Euphorbia ie, Croton, Ricinus and Mercurialis and 6 species were from related families (Geraniaceae, Linaceae and Rutaceae). Being able to complete its life cycle only on plants of genus Euphorbia. O. simplex was approved for introduction into quarantine at Albany, CA. for further testing in late 1980. In quarantine, the lacebug was tested on corn and lettuce and young nymphs were able to complete their development on both the test plants. On corn, nymphal development occurred only on plants which were 12 week old.

In order to have more data to better evaluate the potential impact of this lacebug on corn and lettuce, field experiments were conducted in 1982 at the Rome garden laboratory. The results of these field trials indicated that both corn and lettuce were unsuitable hosts for O. simplex.

Because of the controversial results, obtained under different conditions (ie, laboratory and field), we decided to duplicate the laboratory experiments. The research objectives were: 1) to study the nymphal development of O. simplex on plants of corn and lettuce, which were three weeks old as well as on older plants (6-8 weeks); 2) to ascertain if newly emerged adults, reared on corn and lettuce, were

able to complete the successive generations on the same test plants; 3) to see if newly emerged adults, reared on E. esula, were able to produce eggs when forced to feed on young plants of corn or lettuce (2-3 weeks old) or on older plants (6-8 weeks old).

Nymphal survival test

Thirty adults (16 ♀ 14 ♂) of O. simplex were collected at Piacenza on June 24, 1984, either on the soil in the vicinity of E. esula plants or on the stems of the plant. These adults were brought to the Rome Laboratory and placed in plexiglas cages (10 cm high by 6 cm in diameter), which were provided with bouquets of fresh plants of leafy spurge. Four cages were prepared (4 ♀ 3 ♂/cage). Bouquets were changed twice/week, and those on which oviposition occurred were kept in vials with water and left undisturbed until the emergence of the neonate nymphs. The first nymphal survival test (Test No. 1) was conducted using three week old plants, of sweet corn (Golden Hybrid Blend) and lettuce (Great Lakes variety), as test plants, and on E. esula control plants from Pisa. Newly emerged nymphs were put on bouquets of leaves of the test and control plants held in 200 cc paper cups. Five nymphs/cup were used in each of 10 replicates. Cups were checked twice a week and the food was replaced. Once these insects reached the adult stage, they continued to be confined on lettuce and corn, to ascertain if new adults reared on these plants were able to produce fertile eggs. This test which started on July 13, 1984 and ended on August 6 was conducted in a laboratory room where the temperature ranged between 22°C and 26°C, 50-70%RH and with a natural photoperiod. The second nymphal survival test (Test No. 2) was carried out using older test plants (Sweet corn - Golden Hybrid Blend:

8 weeks old; lettuce (Great Lakes variety: 10 weeks old). Ten newly emerged nymphs/cup were used on each of the 10 replicates. This test, conducted in the quarantine laboratory where the temperature ranged between 15^{o}C and 27^{o}C, was started on August 21 and ended on September 24, 1984.

Results

The data obtained in the nymphal survival tests of O. simplex (Tests No. 1 and 2) gave the following indications:

(Test No. 1)

1) Young plants of sweet corn (three week old) were unsuitable host for O. simplex nymphs.

2) Of 50 neonate nymphs tested with lettuce (3 weeks old), only 7 completed their development, while on the control plant, 43 individuals reached the adult stage.

(Test No. 2)

3) Of 100 neonate nymphs tested with sweet corn (7 weeks old), 27 reached the adult stage. From 100 nymphs tested with lettuce (10 weeks old) and 100 nymphs on the control plant, 64 and 78 individuals survived respectively to the adult stage.

These results are summarized in Table 1.

In the successive phase of the experiment, when the egg production from insects reared on sweet corn and reared on lettuce was determined, the following results were obtained:

1) Adults of O. simplex (4 ♀ 3 ♂), (ex L. sativa, Test No. 1), placed in a plexiglas cage with a bouquet of lettuce, did not produce eggs, but fertile eggs were found on the control plant (E. esula). The females, reared on L. sativa, showed undeveloped ovarioles, when they were dissected, while those from E. esula had 1-2 mature eggs/ovariole.
2) Adults obtained in the test No. 2, ex L. sativa, (10 weeks old) oviposited fertile eggs.

Unfortunately, because of high temperature (45 ^{o}C) occurred in the quarantine, where the test No. 2 was conducted, the majority of the new adults died. Only a few (5 ♀ 6 ♂), ex lettuce survived. For this reason it was not possible to determine if new adults of this lacebug, reared on sweet corn, were able to continue for the successive generation. However the results of these trials indicated that only mature plants of sweet corn and lettuce will support the complete development of O. simplex nymphs. Thus it probably means that mature plants acquire some particular substances which play an important role as nutritional factor for this lacebug.

Oogenesis Test

On July 23, 1984, last instar nymphs of O. simplex, reared on E. esula, were transferred into plexiglas cages provided with young plants of sweet corn (2 weeks old) and lettuce (4 weeks old). For each test plants 2 plexiglas cages were used with 10 mature nymphs/cage. A control plant (E. esula) was also included. The number of nymphs moulting to adult stage was recorded and, the sex, egg production, egg fertility, and adult longevity were also determined. Lastly, after the

females died, they were dissected to determine the development of their ovarioles . The experiment started on July 23 and ended on August 21, 1984 and was conducted in a laboratory room, where the temperature ranged between 20°C 25°C and natural photophase.

Results

Of 20 O. simplex nymphs exposed to young sweet corn, only 10 individuals moulted to the adult stage(6♀ 4 ♂). The new adults survived only a week and were not able to produce eggs. The females, once dissected, demonstrated undeveloped ovarioles. On lettuce, 18 individuals reached th. adult stage (10 ♀ 8 ♂). These insects survived for ten days, no eggs were produced and undeveloped ovarioles were found in the females. Seventeen mature nymphs kept to the (E. esula) control moulted to the adult stage(11 ♀ 6 ♂) and on August 21, the females started to lay fertile eggs.

These results suggest that young plants of sweet corn and lettuce, have a deficiency of nutrients which do not allow the females to get the necessary nutrition for the maturation of the eggs.

Table 1. Results of the nymphal survival tests of *Oncochila simplex* (Test No. 1 and Test No. 2) conducted under laboratory conditions.

TEST PLANTS	No. individuals surviving to: 2nd instar No. (%)	3rd instar No. (%)	4th instar No. (%)	Adults No. (%)
TEST NO. 1[a/]				
Control (*E. esula*, Pisa)	48 (96)	48 (96)	45 (90)	43 (86)
Lactuca sativa (3 weeks old)	38 (76)	23 (46)	14 (28)	7 (14)
Zea mays (3 weeks old)	9 (18)	-	-	-
TEST NO. 2[b/]				
Control (*E. esula*, Pisa)	86 (86)	84 (84)	79 (79)	78 (78)
Lactuca sativa (10 weeks old)	93 (93)	77 (77)	70 (70)	64 (64)
Zea mays (7 weeks old)	69 (69)	52 (52)	39 (39)	27 (27)

a = Five nymphs/cup in each of 10 replicates

b = Ten nymphs/cup in each of 10 replicates

Rome Laboratory

CENTAUREA SOLSTITIALIS (Yellow starthistle)

S. Clement and T. Mimmocchi

Biological Studies of Apion (Coleoptera: Curculionidae) Associated with Centaurea solstitialis L. (Compositae) in Italy and Greece.

A review of the literature indicates that plants in the genus Centaurea (Compositae) in Eurasia are host plants for at least four species of Apion weevils: A. armatum Gestacker, A. penetrans Germar, A. onopordi Kirby, and A. austriacum Wagn. (Hoffmann 1958; Zwolfer 1965 a, b; Scherler 1982; Steklova 1983). Five other Apion species have been associated with Centaurea in unpublished reports written over the last 18 years by researchers at USDA Laboratories in Rome, Italy and Albany, California. The species mentioned in these reports, A. alliariae Herbst, A. carduorum Kirby, A. detritum Rey, A. scalptum Rey, and A. orientale Gerst, were identified by taxonomists at the USDA Systematic Entomology Laboratory, Beltsville, Maryland. All of the aforementioned species are in the subgenus Ceratapion except for A. detritum (subgenus Diplapion) and A. orientale (subgenus unknown to us).

One Eurasian Centaurea that has become a serious pest in western North America is C. solstitialis or yellow starthistle (YST) (see Maddox 1981). This weedy species became a subject for overseas biological control research by USDA, University of California, and C.I.B.C. personnel in the early 1960's, and has been investigated

sporadically, but at the time of this writing no biological control agent (insect or pathogen) from Eurasia has been established in North America. However, some biocontrol workers anticipate that two seedhead insects from Greece, *Urophora sirunaseva* (Hering) (Diptera: Tephritidae) and *Bangasternus orientalis* (Capiomont) (Coleoptera: Curculionidae), will become established in the near future. *Apion* weevils that attack the rosettes of *C. solstitialis* are of interest to researchers working on the biocontrol of this weed because it is important to find an insect that will stress the plant early in the growing season and thus supplement stress caused by insects that are being or will be released to feed on the seeds.

In one of his first reports on the phytophagous insects associated with *C. solstitialis* in Europe, Zwolfer (1965b) indicated that the only insect causing noticeable damage to the rosettes of the plant in southwestern Europe was *A. penetrans*. Zwolfer also reported that adults of this *Apion* fed for several weeks on potted artichoke, *Cynara scolymus*, and cultivated safflower, *Carthamus tinctorius*, and by intense feeding caused heavy damage or killed these test plants; however, this species is not reported in the literature to be an actual pest of these plants. In the *Ceratapion* and other closely related subgenera, *A. carduorum* is the only known pest of a crop plant, and this is artichoke in France (see Poinar et al. 1980). The literature and unpublished USDA reports give *C. solstitialis* as a host plant for three other *Apion* species: *A. scalptum*, *A. alliariae*, and *A. onopordi*. Known host plant records for the *Ceratapion* and *A. detritum* were compiled from various sources and listed previously by us (see 1983 Annual Report of USDA Rome Laboratory).

At this point it is worth stressing that one should not place a lot

of confidence in the identities of any of the aforementioned Apion because there is considerable taxonomic confusion in the Ceratapion and closely related subgenera (Drs. Alonso Zarazaga and D. Whitehead, pers. comm.). For this reason, this report makes no reference to particular Apion species. Zwolfer (1965b), in fact, suggested a checking of the taxonomic position of his A. penetrans.

We initiated a study of the Apion associated with C. solstitialis in central and southern Italy and northern Greece in 1983. Our objectives were 1) to collect information on the life history, host plants, and host specificity of these Apion and, 2) to assemble a large series of Apioninae from Eurasian thistles, especially Centaurea, so appropriate taxonomists could clarify the taxonomy of Apion which use C. solstitialis as a feeding and breeding host.

Study Sites

Six infestations of C. solstitialis were sampled in Italy: Site 1 was a narrow strip (ca. 2m wide x 60 m long) along a secondary road from Via Cassia, 10 km N of Rome, Lazio region (latitude 42° 10', longitude 12° 15'), with a density of $<$80 scattered YST plants in 1983; Site 2 was a roadside area (ca. 1.5 m wide x 20 m long) about 30 km N of Rome and 11.5 km SW of Lake Bracciano, Lazio region (latitude 42° 10', longitude 12° 15'), supporting $<$30 YST plants in 1983; Site 3 was an open 0.7 ha field adjacent to Via Appia Antica, 3 km S of Rome, Lazio region (latitude 41° 50', longitude 12° 45') and YST density, although not estimated in 1983, was 100+ plants; Site 4 was a roadside area (ca. 200 m long) along Hwy 88, 5 km N of Benevento, Campania region (latitude 41° 10', longitude 14° 45') which supported at least 100 scattered YST plants in 1983 and 1984; Site 5

was an open 1 ha field 1.0 km E of Noci, Puglia region (latitude 40° 45', longitude 17° 15') with several hundred YST plants in 1983 and $<$ 100 plants in 1984; Site 6 was an open 3.5 ha field adjacent to Hwy 170, 17 km S of Andria and 0.8 km S of Castel del Monte, Puglia region (latitude 41° 10', longitude 16° 15') which supported several hundred YST plants in 1983 and 1984. These infestations were all sampled in 1983. In 1984, sites 4, 5, and 6 in Italy and two small patches ($<$30 YST plants per patch) of YST within a 10 km radius of Thermi, Greece (latitude 40° 30', longitude 23°) and a roadside infestation (70-80 YST plants) 1.8 km N of Lahanas, Greece (latitude 41°, longitude 22° 45') were sampled. Yellow starthistle (pre-bud formation stages) and competing vegetation at sites 3, 4, 5 and 6 served as food for browsing sheep in 1983. In 1984, sheep again used YST as a food source at site 6.

Materials and Methods

Life History Studies

1983

Ten 1m^2 plots were set out along a transect (ca. 5 m between plots) in the open field at site 6 (Castel del Monte) on June 27, 1983 and all YST plants were removed, placed in numbered plastic bags (one bag/plot), and returned to a laboratory at the USDA Biological Control of Weeds Laboratory, Rome, Italy where each plant was carefully dissected to record the *Apion* larval infestation level. Although larval and pupal development was mostly completed by this date (see Results), it was possible to estimate the number of larvae that each plant had harbored because previous dissections had provided an indication of the type and extent of damage that a known number of

larvae would cause to a plant's stems, crown and root.

For all other 1983 samples, a varying number of YST plants were collected in a random fashion and brought to the Rome laboratory to dissect and record larval infestation levels.

Plants were classified according to the YST growth stages (= phenophases) described in Maddox (1981).

Yellow starthistle infestations were sampled between March 21 and September 30, 1983.

1984

Field Studies

The open field at site 6 was divided into four quadrats of equal size and a random numbers table was used on each sample date (March 14, April 10, May 8, and June 18) to select two quadrats per strata (n = 16). Using a wooden frame (1 m^2), a different sampling area was randomly established in each of the chosen quadrats on each sample date and all YST plants with $\geq$ 7 leaves were removed and placed in numbered plastic bags (one/quadrat). These plants were dissected at the Rome Laboratory to record the number of *Apion* eggs, larvae, and adults and *Apion* parasitoids in each plant. Larvae were preserved in 70% ETOH.

Yellow starthistle plants were placed into distinct growth stages, as follows:

Stage 1 - Late seedling, early rosette stage. Plants with 7-8 leaves.

Stage 2 Early rosette stage; plants with 9-12 leaves.

Stage 3 - Rosette stage; 13-18 leaves.

Stage 4 - Late rosette, pre-bolt stage; $\geq$ 19 leaves.

Stage 5 - Vegetative or beginning bolt stage with stem(s) $\leq$ 2cm in length.

Stage 6 - Bolt stage with stem(s) $\geq$ 3 cm in length.

Stage 7 - Floral bud stages 1, 2, and 3 present.

This scheme is a slight modification of the one developed by Maddox (1981), and was used only for the plant samples from site 6.

Plants were collected outside the stratified plot at site 6 on two dates. Firstly, on May 8, 45 stage 3 and 4 plants showing no evidence of previous sheep damage were removed and transplanted into 22 cm diameter clay pots, and returned to Rome where they were placed under a covered cabana until they were dissected on May 30 to record the stage of development of Apion in crowns and roots. Secondly, on June 19 all YST plants in a 400 m^2 area that had not been damaged by browsing sheep were removed and divided into 2 categories: plants with one stem and plants with more than one stem ascending from the crown. Each plant was dissected in the laboratory to record the extent of the Apion attack in each category of plant. The aim was to determine if a relationship exists between Apion larval feeding in crowns and the formation of multiple stems.

Pupae and larvae were recovered from samples collected in northern Greece. Attempts were made to rear-out the pupae, but larvae were preserved in 70% ETOH. Plants were dissected in a laboratory at the University of Thessaloniki, Agricultural Research Farm, Thermi, Greece.

In 1984, plants were randomly collected at sites 4 and 5, and in Greece. Sampling began March 14 and ended August 22.

Laboratory Development and Host Specificity Studies.

Four female *Apion* weevils were collected on YST rosettes in spring of 1984; one on March 28 at site 4 and three at site 6 between the second and fourth weeks of April. Each female was confined in a 500-cc cardboard container with a young rosette (Italian plants) removed from the garden at the Rome Laboratory. Microscopic examination revealed that these garden plants were free of *Apion* eggs. These rosettes, held in water-filled vials plugged with cotton, were changed 3X per week, and at each change eggs were counted and carefully extracted from the leaves with the aid of fine forceps and a soft camel hair brush, labeled according to female and date collected, and transferred to 35-cc hatching containers (Rizza 1977).

To record the duration of the larval feeding period on YST in the laboratory, we inserted 4 neonate larvae (≤ 8 h old) into a puncture in each of 10 potted rosettes and then dissected the plants at various time intervals. These plants were grown from seed collected near Rome. A steel pin was used to make the puncture in the middle of each rosette and a camel hair brush was used to transfer larvae from hatching containers into punctures. Plants were dissected at 7 (2 plants), 14 (2 plants), 18 (1 plant), 21 (3 plants), 24(1 plant), and 26 (1 plant) days post-larval transfer and larvae collected were placed in 70% ETOH for head capsule measurements. These head capsule measurements were combined with a sample of neonate (≤ 8 hours old) larvae from the hatching containers (n = 27) and larvae from the 1984 plant samples at site 6 (Castel del Monte) in a frequency distribution to determine the number of larval instars.

A small larval survival test was conducted using seven plant species, including three ecotypes of *C. solstitialis*. Table 7 lists

the potted test plants, provides more experimental details, and shows that each plant received one or three neonate larvae. Plants with distinct rosettes received larvae as described above for the larval feeding study on YST. The lettuce plants were infested by placing larvae into small punctures made in the lower part of central leaves. Larvae were placed into punctures made in the midrib of central leaves (2 cm above the crown) of artichoke plants. To successfully transfer larvae to safflower plants, we found it was necessary to place a neonate larva into a well formed puncture made in a stem at a point about 9 cm above the soil surface.

Test plants were dissected 11 to 33 days post-infestation and the number of surviving larvae and pupae was recorded. Larvae were preserved in 70% ETOH so we could measure their head capsules. Larval growth and survival on the test plants was compared with the YST control.

One series of choice and no-choice adult feeding tests was conducted with 35 unsexed Apion collected as teneral adults in the stems, crowns, and roots of YST on June 19 at site 6. Twenty-eight of the adults were fed foliage of YST before tests; seven were starved before they were used in a test. Plastic 1000-cc beakers fitted with nylon organdy covers served as cages. Beetles were checked, feeding damage ratings were assigned for each of the food choices, and fresh bouquets were provided 5, 7, 10, 14 and 17 days after the start of each test. A scale of 0 to 4 was used to assign feeding damage ratings, where: 0 = no feeding; 1 = 1-9 feeding scars; 2 = 10-29 feeding scars; 3 = 30-99 feeding scars; and 4 = 100+ feeding scars. Table 8 provides more experimental details.

Twenty-nine feral Apion weevils, sex ratio unknown, were collected on cultivated safflower (Carthamus tinctorius) growing in pots and in the Rome Laboratory garden and were starved less than 24 hours before being used for another series of choice and no-choice feeding tests. Beetles in cages, as described above, were checked, feeding damage ratings (0 - 4 scale) were assigned to food choices, and fresh bouquets were provided at regular intervals between 3 and 17 days after the start of each test. Table 9 provides more experimental details.

Egg hatching containers and infested potted plants were held in a laboratory room with natural lighting from a window and temperatures of $22 \pm 4^{\circ}C$. Laboratory studies were conducted under the same conditions.

RESULTS

Life History

Adults (collected from YST)

The light black adults are ca. 0.9 mm wide and 1.8-2.9 mm long. Rows of white bristles can be seen on the thorax and elytra of the adults when they are viewed under a microscope.

The four females collected on rosettes in the field in spring 1984 started laying eggs in the laboratory, as shown below.

Female No.	Date of Capture	Date Eggs First Laid in Laboratory
1	March 28, 1984	April 27, 1984
2	April 10, 1984	April 15, 1984
3	April 10, 1984	April 27, 1984
4	April 27, 1984	May 14, 1984

Female 1 laid 39 eggs before she escaped from her container on May 20 and was lost. Female 3 laid 44 eggs before she died on June 15. Females 2 and 4 laid, respectively, 214 and 166 eggs;they were killed in early-August after they stopped laying eggs. These females were not paired with males during their laboratory confinement so mating took place in the field prior to their capture. All of the eggs were viable (see discussion below).

In the field, we have collected adults feeding on the leaves and stems of YST in late-May, June, July, August, and late-September (Table 1 and unpublished information). As far as known, the *Apion* associated with YST in Mediterranean Europe are univoltine with adults overwintering. Our attempts to carry adults through the winter in the laboratory were not successful.

Eggs

Eggs were first found in the field on March 28, 1984 under the epidermis of a root supporting a rosette with about 10 leaves at site 4 (Table 1). The four eggs were inserted into the root between the root neck and down to a distance of 7.6 mm. None of the other 48 plants in the collection had eggs.

Eggs were also recovered from YST rosettes in early-May but these were found on the underside of the leaves, just beneath the epidermis of midribs at the point where leaves were attached to the crown. This oviposition pattern was consistently observed in the laboratory where ovipositing females used their rostrum to make a small shallow cavity in a midrib into which one egg was usually inserted. The oval, smooth eggs were 0.58$\pm$0.03 mm ($\bar{x} \pm$ SD) in length and 0.28 $\pm$ 0.01 mm wide (n = 43). They were light yellow and translucent when laid but gradually

turned black as the embryo developed. A developing larva was visible under the chorion 4-5 days after oviposition. Duration of the egg stage at room temperature (22 $\pm$ 4^{0}C) and natural lighting was 6.36 $\pm$ 1.01 days (n = 14). All of the eggs (n = 463) laid in the laboratory hatched (100% viability).

It is not known if more than one species of Apion is responsible for the eggs laid in roots and leaves of YST. With the reared adult material we now have on hand it may be possible for an Apion taxonomist to resolve this question.

The collective evidence indicates that the 1984 oviposition cycle for Apion on YST in Italy began in late-March and extended a little beyond the first decade of May (Fig. 2; Tables 1 and 3). Two females laid eggs through July in the laboratory so we are not sure what to conclude about the actual time frame for oviposition. Perhaps it indicates the capacity of the species to oviposit, and maybe develop on plants other than YST, but this is speculation until the taxonomy and host range of the YST Apion can be clarified.

Larvae and Pupae

Newly eclosed larvae fed at the base of rosette leaves before moving down to feed within the crown. Older larvae either continued to feed in the crown or they moved down in the root or upwards into a stem. In 1983 in Italy the larval feeding period was mostly completed by mid-June but in 1984 it extended into late-June. Larval feeding was mostly completed by early-June in Greece in 1984 (Table 1).

Stem feeding by Apion in Italy was confined to central pith areas of main stems extending upwards from a crown. Some larval feeding was observed in smaller side stems in Greece but it was not possible to

determine if this feeding was damaging to the plant because of previous browsing by sheep and goats. The only time it appeared that larvae fed upon important structural and vascular tissues in main stems was when as third instars, and just prior to pupation, they chewed an exit hole to the outside. Teneral adults left plants through these holes, which were found at varying distances above crowns; the average distance above crowns for six exit holes was 4.07 ± 2.69 ($\bar{x} \pm$ SD) cm (range 1.7-8.6 cm).

In the laboratory (temperatures of $22 \pm 4^{o}C$), the larval feeding period in artificially infested YST plants was at least 21 days. This was determined by dissecting the plants at specific intervals after egg hatching (Table 2).

Table 1 Summary of results of surveys in Italy and Greece for Apion on Centaurea solstitialis, 1983 and 1984.

Date of Collection	Number of Plants	Plant Growth Stage 2/	Percentage of Plants Harboring Apion and Apion Stages Collected 1/							
			Italy Site No.						Greece	
			1	2	3	4	5	6	Thermi	Lahanas
Mar.21,83	30	Stages 1-3	-	-	0%	-	-	-	-	-
	16	Stages 1-3	0%	-	-	-	-	-	-	-
	10	Stages 1-3	-	0%	-	-	-	-	-	-
Apr. 27,83	43	Stages 2,3	-	-	-	18.6%;L.	-	-	-	-
	101	Stages 1-4	-	-	-	-	-	35.6%;L.	-	-
May 3,83	15	Stages 4,5	33.3%;L.	-	-	-	-	-	-	-
May 18,83	11	Stage 6	72.7%;L,P,A.	-	-	-	-	-	-	-
May 26,83	58	Not recorded	-	-	12.1%;L,P.	-	-	-	-	-
June 14,83	7	Bu-1	14.3%;A.	-	-	-	-	-	-	-
June 27,83	5	Flower-heads	-	-	-	100%;A.	-	-	-	-
June 27,83	31	Flower-heads	-	-	-	-	-	40.7%	-	-
Sept.30,83	ca.100	Senescent	-	-	-	-	A	-	-	-
Mar.14,84	42	Stages 1-3	-	-	-	-	-	0%	-	-
Mar. 28,84	49	Stages 1-3	-	-	-	2%;E.	-	-	-	-
Apr. 10,84	30	Not recorded	-	-	-	0%	-	-	-	-
	120	Stages 1-4	-	-	-	-	-	0%;2°A.	-	-
May 8,84	43	Stage 3	-	-	-		11.6%;E,2L,3L.	-	-	-
	161	Stages 2-6	-	-	-	-	-	26.1%;E,L1-3.	-	-
May 24,84	35	Stage 6 and Bu 1-3	-	-	-	-	-	-	-	25.7%;L2-3,P.
May 31,84	40	Bu 1-3 and Flower-heads	-	-	-	-	-	-	25%;3L,P,A 3/.	-
June 18,84	212	Flower-heads	-	-	-	-	-	25.9%;L3,P,A.	-	-
June 28,84	10	Flower-heads	-	-	-	50%;L,A.	-	-	-	-
Aug.22,84	ca.200	Flower and seed-heads	-	-	-	-	-	A	-	-

1/ E= egg; L= larvae (instar not known); L1, 2 and 3 = first, second and third larval instar; P= pupa; A= adult.

2/ See text for description of growth stages for C. solstitialis.

3/ Data from two sites near Thermi, Greece.

Table 2. Results of dissections of *Centaurea solstitialis* to determine feeding period of *Apion* larvae, laboratory study, Rome 1984.

Days Post-Hatching	No. of Larvae and Pupae Found	Larval Head Capsule Width (mm)	Instar[1/]
7	4	0.32	2L
		0.36	2L
		0.36	2L
		0.40	2L
14	3	0.52	3L
		0.52	3L
		0.52	3L
18	3	0.48	3L
		0.52	3L
		0.52	3L
21	3	0.52	3L
		0.52	3L
		0.48	3L
24	3	Pupa	-
		0.52	3L
		0.52	3L
26	1	Pupa	-

1/ See Fig. 3 for frequency distribution of head capsule widths and larval instars.

The larval head capsule measurements, plotted by frequency (Fig. 1), indicate that there are three larval instars: 0.16 - 0.24 mm (1 L); 0.30 - 0.40 mm (2 L); and 0.44 - 0.56 mm (3 L).

Pupation can take place in the first decade of May, when some oviposition is still occurring. Table 1 shows that during our two year study pupae and teneral adults were extracted from YST between May 18 and June 18. Thus, the new generation of adults first appear around mid-May and they continue to emerge throughout June.

Natural Mortality

Sheep browsing on pre-flowering YST plants may account for some Apion mortality but we have no data to support this hypothesis.

A hymenopterous parasitoid accounted for an unknown level of mortality at site 6 in 1984. Seven parasitoid larvae and pupae, recovered from 5 yellow starthistle plants on June 18, 1984, were reared out and submitted to the USDA Systematic Entomology Laboratory for identification. We speculate that the female of this parasitoid species uses the Apion's exit hole in a YST stem to gain access to host prepupae and pupae. Parasitoid larvae were found feeding on host prepupae and pupae.

Pattern of Attack

In 1984, the Apion at site 4 started to oviposit on YST when a majority of the plants at this location were past the seedling stage (Fig. 2). Eggs were only found on rosettes at sites 4, 5, and 6.

Table 3 provides circumstantial evidence that oviposition continued for a short time beyond May 8 in areas at site 6 where YST plants were generally left untouched by browsing sheep. This can be seen by comparing the infestation level of the May 8 collection, which was found to be 1.39 $\pm$0.78 (sentence continued on page 51)

Fig. 1. Frequency of <u>Apion</u> larval head capsule widths, 1984.

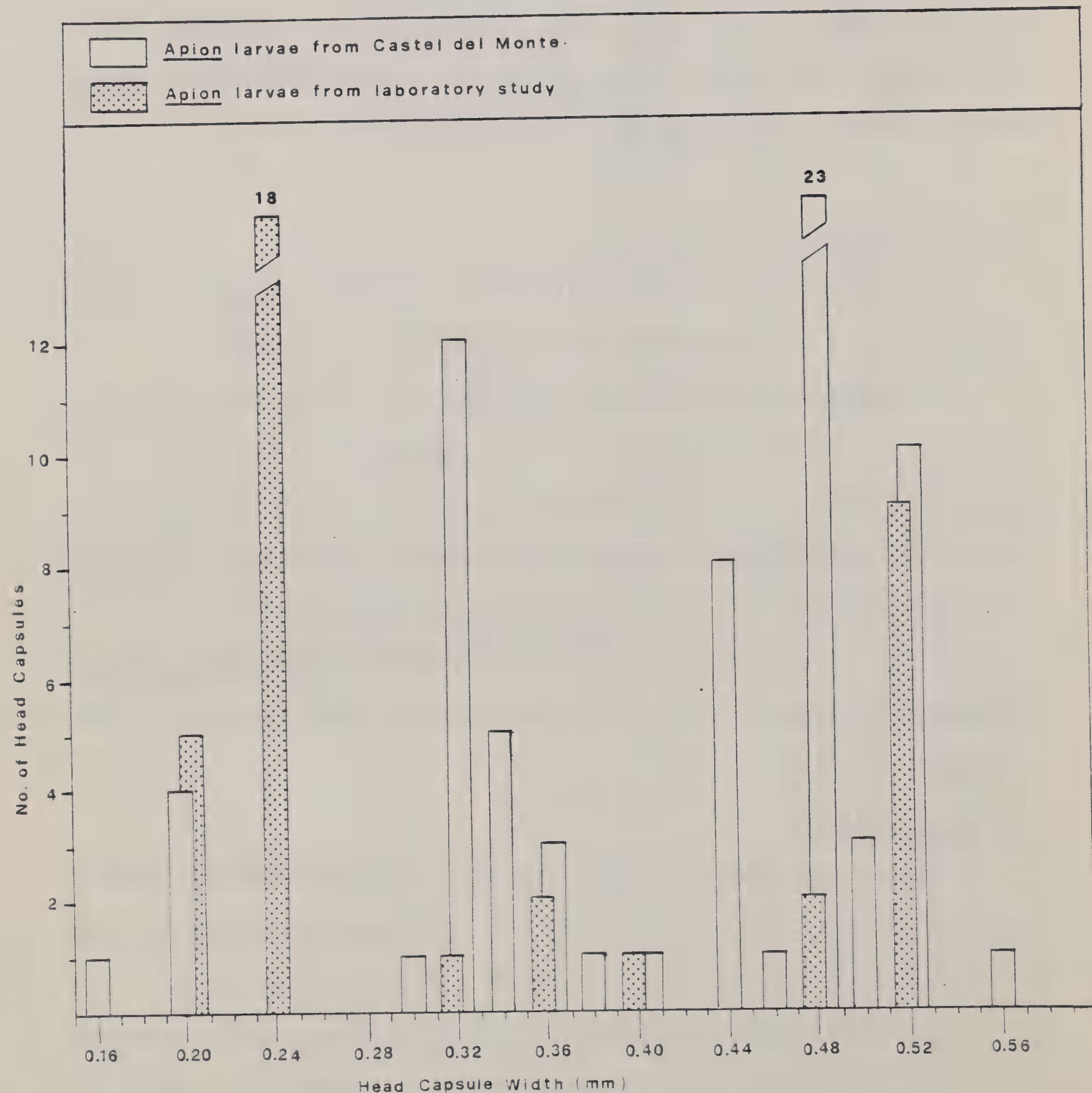

Fig. 2. Apion life history and frequency of growth stages of *Centaurea solstitialis* at Castel del Monte, Puglia region, Italy 1984. See text for description of plant growth stages 1-6. Number of plants per growth stage is shown at top of each histogram.

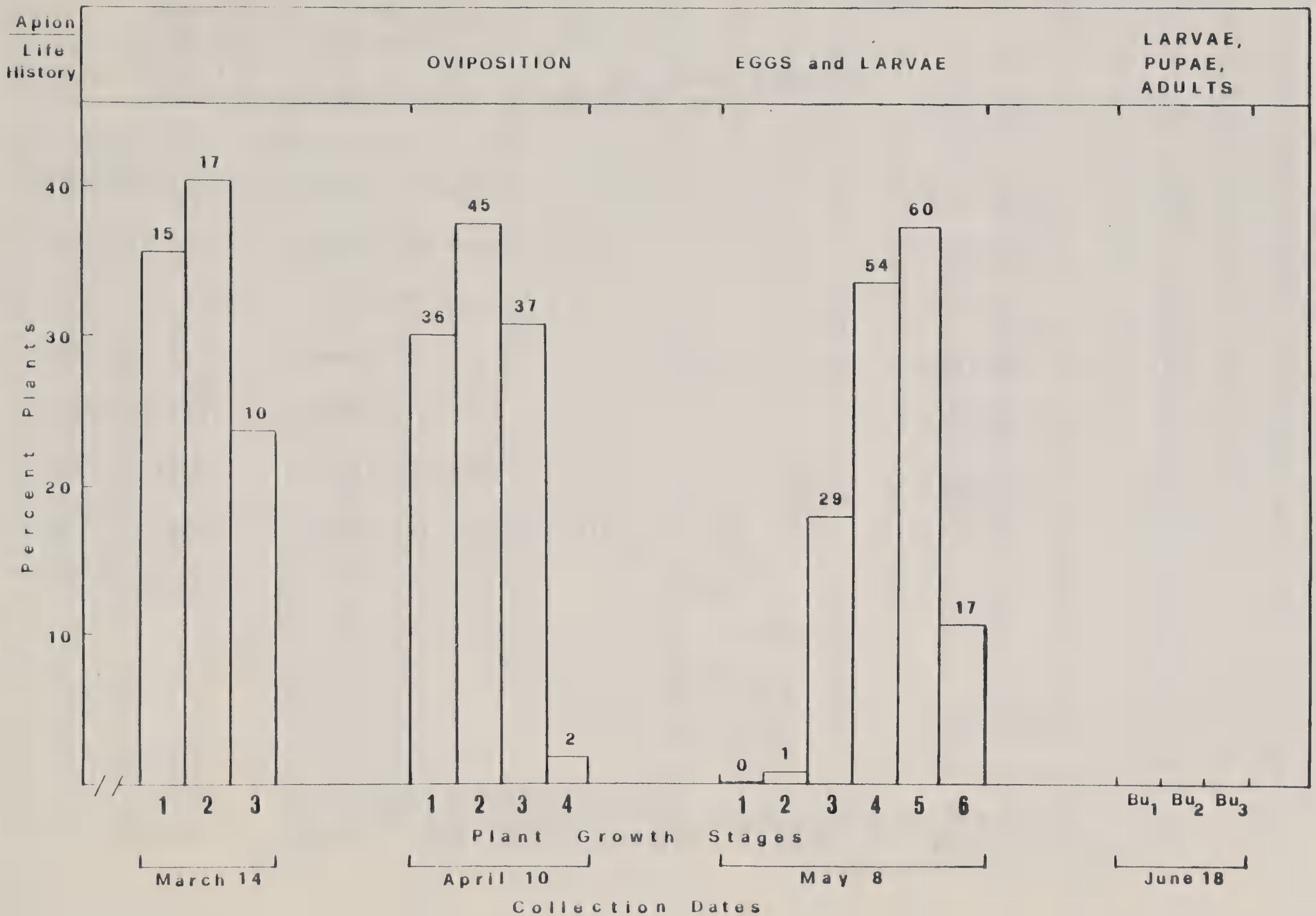

Table 3. Mean (± SD) number of *Apion* larvae in infested plants of *Centaurea solstitialis*, Italy and Greece, 1983 and 1984.

Colletion Date	Site	No. of Infested Plants	$\bar{x}$ ± SD *Apion* per Plant	Range
Apr. 27, 1983	Castel del Monte, Italy	15	1.27±0.46	1-2
May 8, 1984	Castel del Monte, Italy	42	1.24±0.58	1-3
May 8, 1984	Castel del Monte, Italy	18 1/	1.39±0.78	1-4
May 24, 1984	Lahanas, Greece	9	2.78±2.33	1-8
May 31, 1984	Thermi, Greece	10	2.20±1.75	1-5
June 18, 1984	Castel del Monte, Italy	55	1.27±0.68 4/	1-5
June 19, 1984	Castel del Monte, Italy	33 2/	1.79±1.29 4/	1-7
June 19, 1984	Castel del Monte, Italy	23 3/	2.57±1.67 4/	1-8

1/ Plants were removed from the field on May 8, 1984 and placed in pots; they were dissected on May 30, 1984 at the Rome Laboratory.

2/ These were single-stemed plants.

3/ These were multiple-stemed plants.

4/ Because larval and pupal development was mostly completed by mid-June, the number of larvae per plant in these samples was determined from damage criteria as described in the Materials and Methods for 1983 life history studies.

larvae per infested plant, with the higher levels recorded for the two June 19 samples from the same site. A Model II ANOVA analysis for unequal sample sizes (Sokal and Rohlf, 1969) generated a significant (F = 4.30; $P < 0.01$) added variance component among these three groups, with 0.235 being the proportion of variation among the group means..

The YST plants in Italy averaged between 1.24 and 2.57 Apion larvae per plant; averages were 2.20 and 2.78 in northern Greece (Table 3). The two Greek samples and the June 19 sample of multiple-stemed plants from site 4 in Italy contained more plants with two or more Apion, which is reflected by the higher variance (SD) component around the mean ($\bar{x}$) of each sample (Table 3). The fact that infested plants usually harbored one or two larvae can be seen if the infested plants from the 1983 and 1984 collections at site 6 are categorized according to various larval infestation levels (Tables 4 and 5).

The Apion were randomly distributed in the YST population at Castel del Monte in June of 1983 and 1984. This conclusion was reached because the x^2 statistic was 10.94 ($P > 0.05$) and 19.48 ($P > 0.05$) in 1983 and 1984, respectively. Perhaps this Apion followed a random pattern of attack on YST at Castel del Monte? The variance to mean ratio was tested for departure from randomness with the x^2 test (x^2 (n-1 df) = s^2 (n - 1)/$\bar{x}$) (Elliot 1977) because this ratio is a simple index of aggregation, being easy to compute and readily understandable.

Plants removed on June 19, 1984 from the 400 m^2 area at site 6 where little or no sheep browsing had occurred were divided into two categories, single or multiple-stemed, and dissected to record the presence or absence of Apion feeding in the crowns and roots of these plants. A contingency table (Table 6) was constructed and the X^2

statistic was 2.84 (X^2 0.05, 1 d.f. = 3.84), leading us to accept the null hypothesis that the category of plant was independent of the presence or absence of *Apion* larvae feeding within crowns and roots.

Table 4. The number of *Apion* infested plants of *Centaurea solstitialis* in each infestation category, Castel del Monte (Puglia), Italy, June 27, 1983.

No. Infested Plants	Infestation Category (No.of *Apion*)			
	1-2	2-3	3-4	4-5
33[1/]	22	8	2	1

1/ Plants were removed from ten 1m^2 plots (ca. 5 m. between plots).

Table 5. The number of *Apion* infested plants of *Centaurea solstitialis* in each infestation category, Castel del Monte (Puglia), Italy, 1984

Date Collected	Plot No.	Infestation Category (No. of *Apion*)				
		1	2	3	4	5-9
May 8	1 1/	35	4	3	0	0
June 18	1	44	9	1	0	1
June 19 3/	2 2/	19	8	3	2	1
June 19 4/	2	7	6	5	3	2

1/ Plot 1 was 400 x 200 m in size.

2/ Plot 2 was 20 x 20 m in size and adjacent to plot 1.

3/ Plants rated in this row had one main stem.

4/ Plants rated in this row had 2-7 main stems

Table 6. Contingency table for the number of single and multiple-stemed plants of *Centaurea solstitialis* with and without *Apion* larvae, June 19, 1984, Castel del Monte, Italy.

Plant Condition or Category	Attribute		
	With *Apion*	Without *Apion*	Totals
One stem	33	18	51
2-7 Stems	23	25	48
Totals	56	43	99

Laboratory Host Specificity Studies

Apion larvae developed to third instars and pupae on the 3 ecotypes of yellow starthistle and cultivated safflower (Table 7). They were unable to develop on *Centaurea calcitrapa*, *Cirsium douglasii*, *C. campylon*, *Cynara scolymus*, and *Lactuca sativa*. However, we may have inadvertently increased the chances for larvae to survive and develop on safflower because neonate larvae were placed deep (close to the center) into stems. Another point worth mentioning is that *Apion* females are not likely to make deep cavities in host plant tissue for placement of eggs. Eggs in the field and laboratory were all placed in shallow incisions in leaves and roots. It is clear that more larval survival tests are necessary to clarify the capacity of YST *Apion* to develop on various plants.

Significantly ($P < 0.05$) more adult feeding took place on yellow starthistle when adults reared from this plant were given a choice between it and safflower (Table 8). However, weevils from YST readily fed on safflower buds under no-choice conditions; the mean damage rating for weevils on YST buds (2.30 ± 1.14; n = 7) and safflower buds (2.40 ± 1.14; n = 5 - 6) were similar (Table 8).

Table 7. Summary of results of larval survival tests with Apion, Rome, Italy, May 14 - July 1, 1984.

Test Plants and Source of Seed	Growth Stage and Size of Test Plants that Received Larvae	Test 1[1] No.Plants Tested	Test 2[2] No. Plants Tested	No.Plants with Surviving Larvae/Pupae at Dissection	Apion Stages Recovered at Various Dissection Times Post-Larval Infestation
Centaurea solstitialis	Rosette, spread 10 cm	6	-	6	one 3L/Plant (12-21 days)[3/4/]
Bracciano, Italy	Rosette, spread 13 cm	-	3	3	one P/Plant (33 days)
C. solstitialis	Rosette, spread 15-23 cm	4	-	3	one 3L/Plant (21 days)
Contra Costa, CA., USA	Rosette, spread 25-30 cm	-	3	3	two or three P/Plant (29 days)
C. solstitialis Walla Walla, WA., USA	Rosette, spread 24 cm.	1	-	1	one P(24 days)
Centaurea calcitrapa	Rosette, spread 10-13 cm.	4	-	0	(21 days)
Noci, Italy	Rosette, spread 12-15 cm.	-	3	0	(28 days)
Carthamus tinctorius Hartman Variety, USA	Seedlings, 6-9 leaves, height 13-20 cm.	6	-	3	one 3L/Plant (11-21 days)
	Flowering, height 50-60 cm.	-	3	1	one 3L (29 days)
Cirsium douglasii CA, USA	Rosette, spread 10-15 cm., 5-7 leaves	3	-	0	(21 days)
	Rosette, spread 40-50 cm., 15-20 leaves	6	-	0	(21 days)
	Rosette, spread 20 cm., 5-6 leaves	-	3	0	(30 days)
Cirsium campylon CA, USA	Rosette, spread 25-30 cm., 10-15 leaves	-	2	0	(23 days)
Cynara scolymus Green Globe Cultivar, USA	2nd year plants, height 20-25 cm., 4-5 leaves	5	-	0	(21 days)
	2nd year plants, height 25-30 cm., 5-6 leaves	-	3	0	(28 days)
Lactuca sativa NK-Bibb, USA	Width 7-10 cm., height 3-4.5 cm.	4	-	0	(21 days)
	Width 10-12 cm height 7 cm.	-	3	0	(28 days)

1/ Test 1 plants each received one newly hatched larva.

2/ Test 2 plants each received three newly hatched larvae.

3/ 3L = 3rd Instar Larva; P = Pupa.

Table 8. Summary of results of choice and no-choice laboratory feeding tests with adult Apion reared from Centaurea solstitialis (YST), Rome, Italy, June 26 - July 13, 1984.

Test	No. Adults in Test 2/	Food Choices 3/	Feeding Damage Rating 1/ at Days After Start of Test: 5	7	10	14	17	$\bar{x}$ (+SD) Feeding Scar Values per Food Choice
Choice 1	7 4/	YST buds	4 (7) 6/	3 (7)	4 (7)	3 (7)	2 (7)	3.20+0.84a 7/
		YST leaves	4	2	3	3	2	2.80+0.84a
		Safflower bud	0	1	0	0	0	0.20+0.45b
Choice 2	7 5/	YST buds	4 (7)	4 (7)	2 (7)	2 (7)	1 (7)	2.60+1.34a
		YST leaves	4	4	4	2	2	3.20+1.10a
		Safflower bud	2	0	1	0	0	0.60+0.89b
No-Choice 1	7 5/	YST buds	4 (7)	3 (7)	3 (7)	2 (7)	1 (7)	2.80+1.14
No-Choice 2	7 5/	Safflower buds	1 (6)	4 (6)	3 (5)	2 (5)	2 (5)	2.40+1.14
No-Choice 3	7 5/	No Food or Water	- (5)	- (3)	- (0)	-	-	

1/ Based on a scale from 0 to 4: 0 = No feeding; 1 = 1-9 feeding scars; 2 =10-29 feeding scars; 3 = 30-99 feeding scars; 4 = 100+ feeding scars.

2/ Beetles came from plants collected June 19, 1984 at Castel del Monte, Italy.

3/ Food choices were 2-3 YST buds (Stages Bu2-3), 1-2 YST leaves from rosettes, and one safflower bud 10-12 mm in diameter. Material was obtained from potted plants.

4/ Beetles were starved 2.5 - 5 days before start of test.

5/ Beetles were allowed to feed on YST leaves from rosettes for 2.5-5 days before start of test.

6/ Number in parentheses indicate number of beetle alive on each day.

7/ Numbers in a column within a test followed by the same letter are not significantly different (P = 0.05), as determined by analysis of variance and Duncan's multiple range test. Data were transformed by $\log_{10}(x + 1)$ before analysis, but original data are reported. F = 40.41 for Choice Test 1 and F = 9.51 for Choice Test 2.

Table 9. Summary of results of choice and no-choice laboratory feeding tests with adult Apion collected on Carthamus tinctorius, Rome, Italy, June 26 - July 19, 1984.

Test	No. Adults[2/] in Test	Food Choices[3/]	Feeding damage rating[1/] at days after start of test								$\bar{x}$ (± SD) Fee[illegible] Scar Values [illegible] Food Choice
			3	5	7	10	13	14	16	17	
Choice 1[4/]	7	Safflower bud	-	4 (7)[6/]	4 (7)	4 (6)	-	3 (6)	-	1 (6)	3.20±1.30 [7/]
		YST buds	-	0	0	2	-	1	-	1	0.80±0.84
		YST leaves	-	2	3	0	-	1	-	0	1.20±1.30
No-Choice 1[5/]	7	YST buds	3 (7)	-	2 (7)	1 (7)	1 (7)	-	2 (7)	-	1.80±0.84
No-Choice 2[5/]	8	Safflower bud	4 (8)	-	4 (8)	4 (8)	4 (8)	-	2 (8)	-	3.60±0.89
No-Choice 3[5/]	7	No Food or Water		- (7)	- (5)	- (5)	- (0)	-	-	-	-

1/ Based on a scale from 0 to 4: 0 = No feeding; 1 = 1-9 feeding scars; 2 = 10-29 feeding scars; 3 = 30-99 feeding scars; [illegible] 100 feeding scars.

2/ Beetles were collected on Carthamus tinctorius growing in pots and in a garden at the Rome Laboratory.

3/ Food choices were 2-3 YST buds (Stages Bu2-4), 1-2 YST leaves from rosettes, and one safflower bud 11-15 mm in diame[illegible]. Material was obtained from potted plants.

4/ Beetles for this test were collected on June 25, 1984 and held overnight without food before test was started on June 26.

5/ Beetles for these tests were collected on July 3, 1984, when tests started.

6/ Numbers in parentheses indicate number of beetles alive on each day.

7/ Values provide an indication of the amount of feeding over time on each food choice. Note that one beetle died between the 7th and 10th day of the test.

The beetles collected on cultivated safflower showed a weak preference for safflower buds over YST buds and leaves in an adult feeding choice test (Table 9). Under no-choice conditions, weevils from safflower fed more on their source plant (3.60 ± 0.89 average feeding rating over 17 days) than they did on YST buds (1.80 ± 0.84); however, the value for safflower was generated from 8 weevils, whereas the value for YST buds came from 7 weevils.

All the beetles died after 10-13 days in tests where food and water were absent (Tables 8 and 9).

Concluding Remarks

Taxonomic studies are needed before we can determine the taxonomic relationships and exact larval and adult host ranges of the *Apion* associated with *C. solstitialis* in Mediterranean Europe and Turkey. Other investigations should address female oviposition under choice and no-choice laboratory conditions and broad surveys are necessary to record the presence or absence of the insect from plants related to the target weed (Zwolfer and Harris, 1971, state that a field survey is the best single index of host specificity). Lastly, a relationships should be established between *Apion* larval and adult feeding and damage to *C. solstitialis* in Italy, Greece and Turkey.

We have not observed *Apion* inflicting serious damage to *C. solstitialis*, although we are aware of Zwolfer's (1965b) early observation that *Apion* caused noticeable damage to rosettes. Moreover, Rosenthal and Andres (pers. comm.) have indicated that *Apion* may damage or stress the target weed in Turkey.

If the taxonomy of the YST *Apion* is straightened out, with substantiation of a limited and acceptable natural host range for a

species, and a strong association is established between the insect's feeding and damage to YST, then we may yet have a promising biocontrol agent in the genus *Apion*.

The [illegible]

[illegible]

[illegible]

[illegible]

[illegible]

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Yellow Starthistle Garden

Clement, Mimmocchi

A Latin square design was used for yellow starthistle gardens in 1983 and 1984. There were 6 and 8 treatments, respectively, in 1983 and 1984 with one plant per block. The distance between plants was 1 m in 1983 and 1.5 m in 1984. A distance of 50 m separated the 1984 plot and a planting of several hundred yellow starthistle plants (Italian ecotype). Other details of the 1983 work were provided in the 1983 Annual Report.

The flowerheads (stage F-2 according to Maddox 1981) on each plant were collected at 5 to 14 day intervals between July 15 and October 22 in 1984 and placed in cardboard containers fitted with nylon organdy covers so emerging seedhead insects could be collected and mounted up. Containers were held in a laboratory room for emergence.

We are not prepared to write a full account of the results from the 1983 and 1984 YST gardens because identities have not been obtained for most of the several hundred insects that have emerged from the seedheads. Moreover, it will take a few months to finish dissecting the seedheads from the 1984 plot.

Research objectives for this study are: 1) To determine if Italian biotypes of the trypetid flies, _Urophora sirunaseva_ (Hering) and _U. quadrifasciata_ (Meigen) will find, attack and complete their development in the heads of different ecotypes of yellow starthistle. 2) To determine the overall diversity and pattern of attack of the natural enemies of yellow starthistle in central Italy. 3) To determine the variance in herbivore load between and within ecotypes of _C. solstitialis_. 4) To generate baseline information on the plasticity of certain characters of _C. solstitialis_ ecotypes grown under uniform conditions.

Table 1 lists the treatments for the 1983 and 1984 plots and the seedhead production of the various ecotypes. The results reveal considerable variability.

Table 1. Seedhead production of _Centaurea solstitialis_ (YST) ecotypes grown in garden plots, Rome Laboratory, 1983 and 1984.

Ecotype or Treatment	1983[1/] No. Seedheads		1984[2/] No. Seedheads	
	$\bar{x} \pm$ SD	Range	$\bar{x} \pm$ SD	Range
Italian YST (Control)	363.0±350.8(5)[3/]	34-773	339.0±181.1(8)[3/]	145- 602
Spanish YST	152.5± 80.4(6)	31-261	566.0±163.1(8)	293- 761
Concord, CA. YST	208.0±142.0(4)	35-334	-	-
Tehama, CA. YST	237.7±138.3(6)	80-392	-	-
Sacramento, CA. YST	-	-	738.6±222.9(8)	395-1,057
Contra Costa, CA. YST	-	-	417.3±118.4(8)	221- 608
Walla Walla, Wash.YST	108.2±115.6(6)	25-303	285.0± 86.6(8)	185- 416
Yakima, Wash. YST	278.7±180.2(6)	73-593	573.5±349.0(8)	112-1,146
Lapuai, Idaho, YST	-	-	338.6±138.5(8)	64- 491
Carthamus tinctorius	not grown		Grown but data not available	

1/ Plants started from seed on January 31 in a greenhouse and transplanted to plot on March 25.

2/ Plants started from seed on or about February 3 in a greenhouse and transplanted to plot on April 3.

3/ (n)

Tebenna sp. (Lepidoptera: Chorentidae)

We collected additional life history and host plant information on Tebenna sp. (prob. micalis)(Lepidoptera: Choreutidae), a small moth (wing span about 7-8 mm) that breeds on YST. This insect occurred in low numbers on yellow starthistle in the Rome garden in 1983. Dr. Hodges (USDA Systematic Entomology Laboratory, Beltsville, MD) identified this moth from specimens submitted in 1983.

This insect was much more abundant on yellow starthistle in the Rome garden in 1984. Eggs were laid and larvae completed their development on plants from each of the seven YST ecotypes represented in the Rome Latin square plot. The larvae mined basal leaves and the winged stems. There were two or more generations during the summer season. One Yakima YST was heavily infested (20 larvae) in early-July and this had the effect of delaying bolting and formation of buds; however, the plant still produced 915 seedheads.

CENTAUREA SOLSTITIALIS (Yellow starthistle)

P. Dunn, G. Campobasso, F. Murano (part-time)

1) Bangasternus orientalis Capiomont (Col: Curculionidae)

At the beginning of October 1983, seeds of 29 species of test plants were planted in a greenhouse, but problems, such as poor germination, and synchronization with the oviposition period of the weevil (the adult needs to oviposit in the immature flower head) drastically reduced the number of plants suitable for testing. Only 12 plant species were available this year for the experiments.

Two hundred seventy (270) active adults of B. orientalis were field collected in Kozani (Greece), and sent to the Rome Laboratory. These insects were used in a "no-choice" oviposition test and a larval survival test at the Rome Laboratory during May and June.

Material and Methods

Experiments:

No-choice oviposition test: This test was carried out in the quarantine facility, using potted plants (T 15-30 ^{0}C; RH 30-70%, and a photoperiod of 16 hours). The following plant species were used in the test: Centaurea solstitialis (Greece, control), Centaurea paniculata (Italian), Centaurea calcitrapa (Greece), Carduus thoermeri (USA), Carduus nutans (USA), Carduus acanthoides (USA), Cynara scolymus (USA), Lactuca sativa (USA), Onopordum illyricum (Italian), Artemisia vulgaris (Italian), Viola ciocca (Italian), and Carthamus tinctorius (USA). Each plant was caged in a transparent plastic cylinder (diam 20 cm; height 70 cm) with four organdy covered holes (10 cm diameter) on

the sides to permit air circulation, and capped with organdy cloth held in place by a large rubber band. On 2 June, 2 ♀ and 2 ♂ weevils were caged with each of 5 replicates of each test plant species. The plants were dissected and examined on July 24, after most of the adults had died. The results are presented in Table 1.

Egg production: To obtain eggs for a larval survival test, ten (10) pairs of *Bangasternus orientalis* were caged with *Centaurea solstitialis* plants in the quarantine greenhouse on June 5. Every day, newly laid eggs were removed and put in hatching containers. Two days before hatching, when the head capsule was visible through the chorion, the mature eggs were transferred to test plants.

Larval survival test: The same plant species used in the no-choice oviposition test were used for this test which was conducted in the quarantine greenhouse under the same conditions as the preceding test. One bud on each of the 10 replicates of each species tested was infested with 2 fertile eggs (total of 2 eggs/plant = 20 eggs/plant species tested). Eggs were transferred from the hatching containers with a fine brush, and placed between bracts of the immature buds of the test plants. Each infested bud was marked with a label and the date the egg was placed on the plant making it easy to check the eggs for eclosion. All of the eggs hatched within 3 days of transfer. Because mature eggs were available at different times the replications were set up at different times but each test plant was dissected 35 days after that particular replicate was infested. Results are summarized in Table 2.

Results and Discussions

The results, presented in Table 1 and 2 are favorable for the introduction of B. orientalis. Oviposition and survival occurred only on the genus Centaurea. The two eggs found on Centaurea paniculata hatched but the larvae were not able to develop; they both died in the first instar after making small holes in the bracts of the flower bud. The finding of one second instar larva in a Centaurea calcitrapa head was not really a surprise because a "biotype" of Bangasternus orientalis was found feeding and ovipositing on C. calcitrapa in Greece in 1982. The results from three years of experimentation (1982-1984) showed that B. orientalis easily accepted the various U.S. biotypes of Centaurea solstitialis and never accepted plants of economic importance which were offered. From the evidence developed in our trials the host range of the weevil seems to be restricted to the genus Centaurea.

Table 1. "No-choice" oviposition test (plant dissected after 53 days)

Replicate No.:	1	2	3	4	5	Total eggs
Test plants						
Centaurea solstitialis (Greece) Control	411	59	126	139	174	909
Centaurea paniculata	0	0	2	0	0	2
Carduus acanthoides (USA)	0	0	0	0	0	0
Carduus nutans	0	0	0	0	0	0
Carduus thoermeri (USA)	0	0	0	0	0	0
Cynara scolymus (USA)	0	0	0	0	0	0
Lactuca sativa (USA)	0	0	0	0	0	0
Carthamus tinctorius (USA)	0	0	0	0	0	0
Artemisia vulgaris	0	0	0	0	0	0
Onopordum illyricum	0	0	0	0	0	0
Viola ciocca	0	0	0	0	0	0

Table 2. First instar larval survival test (Plants dissected 35 days after infestation

Replicate No.:	1	2	3	4	5	6	7	8	9	10	
Test plants	No. larvae alive at dissection										Total larvae alive
Centaurea solstitialis (Greece) control	1	0	2	0	1	1	0	1	0	1	7
Centaurea calcitrapa (Greece)	0	0	0	0	0	1	0	0	0	0	1
Centaurea paniculata	0	0	0	0	0	0	0	0	0	0	0
Carduus acanthoides (USA)	0	0	0	0	0	0	0	0	0	0	0
Carduus nutans (USA)	0	0	0	0	0	0	0	0	0	0	0
Carduus thoermeri (USA)	0	0	0	0	0	0	0	0	0	0	0
Cynara scolymus (USA)	0	0	0	0	0	0	0	0	0	0	0
Lactuca sativa (USA)	0	0	0	0	0	0	0	0	0	0	0
Carthamus tinctorius (USA)	0	0	0	0	0	0	0	0	0	0	0
Artemisia vulgaris	0	0	0	0	0	0	0	0	0	0	0
Onopordum illyricum	0	0	0	0	0	0	0	0	0	0	0
Viola ciocca	0	0	0	0	0	0	0	0	0	0	0

2) Cyphocleonus morbillosus

For the second year investigations on the root feeding weevil Cyphocleonus morbillosus were conducted in the Rome Laboratory. On May 16, 1984 a field trip in South Italy (Salerno) was made revisiting a known Cyphocelonus collection site. One hundred fifty (150) rosettes of C. solstitialis were examined in the field and only 1 female was found. The insect was brought back to the Rome Laboratory where it was caged with the host plant Centaurea solstitialis.

Materials and Methods

Egg production: In order to provide the opportunity for the field captured female of C. morbillosus to oviposit, it was caged with potted Centaurea solstitialis rosette in a clear, plastic cylinder cage (diam 20; height 70 cm) with four organdy covered holes (diam 10 cm) on the sides (to permit air circulation) and capped with organdy cloth held in place by a large rubber band. The plant was changed daily and the plant which had been exposed to the weevil was carefully checked, recording the oviposition site, number of eggs, and any damage done to the host. The test was carried in the laboratory quarantine greenhouse with temperature ranging between 15-30 ^{o}C; RH 40-85%; and natural day length (15 hours).

Laboratory colony: On May 21, with first eggs were obtained from the ovipositing female a laboratory colony was started on potted C. solstitialis rosettes (Salerno biotype) in which each plant was infested with one fertile egg. Eggs were placed between leaf bracts

and checked daily until eclosion. In order to avoid attack by other insects that naturally damage Centaurea solstitialis rosette in the Rome area and which compete with C. morbillosus larvae, the infested rosettes were initially kept in quarantine under the following climatic conditions: Temperature 15-30 ^{o}C; RH 40-80% and a 16 hour day length. After fifteen days all the infested plants were moved outside in the laboratory garden to natural conditions. The trial lasted from May 21 until August 25.

Preliminary choice test: With 5♀♀ obtained from the laboratory colony a preliminary test was made using the two crop plants, Cynara scolymus and Beta vulgaris var. saccariphera and a Centaurea solstitialis (Salerno biotype) control. All 3 plants were transplanted together in a 35 cm diameter pot, and caged under a transparent cylinder (diameter 30 cm, height 60 cm). Once a week the plants were checked, recording the feeding damage. The experiment was started and kept in the laboratory garden under natural conditions from August 24 to October 1, and later because of bad weather conditions it was moved to a greenhouse until November 1.

Because of the paucity of meaningful data and problems encountered during two years of research on C. morbillosus we decided to stop working with this insect. If the need for a root feeding natural enemy of yellow starthistle seems a necessity in the future, work may be resumed.

Results and Discussions

The single field collected female of C. morbillosus laid 74 eggs, of which 70 were fertile. Eggs were normally laid in groups of 2-3 on the mid vein of the leaf (lower part) from 2 to 5 cm above the root crown and the length of the oviposition period was about 21 days.

The crop Cynara scolymus was heavily damaged by C. morbillosus adults (6800 mm^2 consumed) but no feeding was noticed on Beta vulgaris variety saccariphera and only 250 mm^2 of the control was consumed.

	1983	1984
Ovipositional site:	crown	mid vein (lower part)
Fecundity:	170 eggs	74 eggs
Length of oviposition	48 days	21 days

Because of the [illegible] encountered data and problems [illegible]

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working with [illegible]. It [illegible] for [illegible] most feasible naturally

[illegible] yellow [illegible] characteristic [illegible] [illegible]

CENTAUREA DIFFUSA (Diffuse knapweed)

P. Dunn, G. Campobasso, F. Murano (part-time)

1) Bangasternus provincialis Fairmaire

In 1980 research on the biological control of Centaurea solstitialis was re-activated, and was divided between the three laboratories; Rome, Albany and Thessaloniki. Maddox at Albany developed a list of possible candidates for the biological control of C. solstitialis. One of the insects proposed was the weevil Bangasternus provincialis which attacks immature flower heads of C. diffusa and C. solstitialis. Sobhian, (personal communication) considers C. solstitialis to be a marginal host of B. provincialis. Results obtained from a field experiment in Greece in 1984, by Dr. Sobhian and Mr. Maddox, revealed that the host range of B. provincialis is restricted to the genus Centaurea, with no eggs or feeding damage being found on the two crop plants included in the test; Carthamus tinctorius and Cynara scolymus. In addition, no negative information was turned up in the literature search conducted at Rome.

Since the preliminary research on B. provincialis has been positive so far we are planning to continue in the summer of 1985. The test plant list will be enlarged to include several ecotypes of diffuse knapweed and North American cultivars of Carthamus tinctorius and Cynara scolymus.

Also, in order to try to find cold hardy biotypes of B. provincialis, surveys will be made in the areas of Greece climatically more similar to the knapweed infested areas of N. America than Thessaloniki, where the insect was found and the preliminary testing

was done. In addition we will try to collect from areas where yellow starthistle is not present thus have a greater possibility of getting a biotype of B. provincialis specific to knapweed.

2) Pterolonche inspersa Stgr. (Lepidoptera: Pterolonchidae)

In 1984 the screening experiments with the root feeding larvae of P. inspersa were completed at Rome. On July 6, 1984 infested roots of Centaurea diffusa containing mature larvae of P. inspersa were collected in Greece and shipped to the Rome Laboratory where they were kept until adults emerged. These adults were used for "producing eggs", which provided larvae to complete the screening trials, and provided fertile eggs which were sent to Albany, California to be used in their quarantine testing program.

Pterolonche inspersa Rearing: The two hundred infested roots of C. diffusa received from Greece were transplanted in 3 thirtyfive cm diameter terra cotta pots containing sandy river soil. Each pot was covered by a transparent plastic cylinder with four organdy covered holes (15 cm diam) on the sides to permit air circulation. Each tube was capped with organdy cloth held in place by a large rubber band and kept in the quarantine greenhouse where the ambient temperature and humidity ranged between 15-35^{o}C, RH 40-80% and the photoperiod was ca 16 hours. Plants were lightly watered only when the soil became very dry. Overwatering can seriously compromise the insect emergence.

Egg Production: The emerging adults of *P. inspersa* were collected daily from the rearing cages, sexed, then transferred in to six screen covered cages (90 x 90 x 90 cm) located in the garden, each containing nine (9) potted rosettes of *C. diffusa*. Ten (10) ♂♂ and 10 ♀♀ were used in each cage. To furnish food for the adults, a honey solution (5%) was sprayed on the walls of each cage twice a day at 0800 and 1500 hours. The potted plants used in the trial were exposed to the ovipositing adults for 10 days and then removed and checked for eggs under a stereoscopic microscope. During the oviposition period the outside temperature ranged between 10-35°C, RH 40-85% and the day length was about 16 hours.

All eggs found were removed with a fine brush and transferred to hatching containers (plastic cup with a plaster of Paris substrate) until eclosion. No water was used in the hatching containers during the incubation period.

Adult Oviposition Behavior: In order to establish the time of day the adult of *P. inspersa* started to lay eggs, an experiment was set up in the garden starting August 24 at 1100 and terminating August 25 at 1300 hours.

Five potted plants of *C. diffusa* were each caged with 1♂ and 1♀ *P. inspersa* adults 2 days old. The cage was a transparent cylinder (diam. 20 cm; height 70 cm) with four holes to permit air exchange, and capped with organdy cloth held in place by a large rubber band.
Every two (2) hours each pair of moths was changed to a new cage with an egg free potted plant of *C. diffusa*.
Each removed potted plant which had been with the insects was examined under a stereoscopic microscope and any eggs found were counted, removed and recorded.

Table 1. Time table of ovipositing female of *P. inspersa*.

Time	No. Eggs Laid
1100-1300	0
1300-1500	12
1500-1700	8
1700-1900	15
2100-2300	24
2300-0100	21
0100-0300	13
0300-0500	20
0500-0700	1
0700-0900	0
0900-1100	3
1100-1300	0

Experiments

First instar larval survival test: The following plant species were used for this experiment. *Centaurea diffusa* (Greece control), *Centaurea paniculata*, *Centaurea maculosa*, *Acroptilon repens*, *Daucus carota*, *Beta vulgaris* (var. *saccariphera* USA), *Beta vulgaris* (var. *saccariphera* Italy), *Beta vulgaris* (var. *alba*), *Beta vulgaris* (var. *rubeus*), *Asparagus officinalis*.

The test was conducted in the quarantine greenhouse under the following conditions: Temperature 15-25 ^{o}C; RH 40-80% and a photoperiod of 16 hours. Five (5) potted plants (replicates) of each species were each infested with 2 fertile eggs and 1 newly hatched first instar larva. Each egg position on test plants including the control was marked in order to observe the egg through eclosion in order to be sure the eggs hatched. The test lasted from July 31 until October 2, when all the plants were dissected under a stereoscopic microscope and the surviving larvae were counted, collected and stored in ethyl alcohol. Results are summarized in table #2.

Table 2. First instar larval survival test (Plants dissected 63 days after infestation)

Replicate No.	1	2	3	4	5	
Test Plants	No. Larvae Alive at Dissection					Total larvae alive
Centaurea diffusa (Greece control)	0	1	1	1	1	4
Centaurea paniculata Italy	0	0	0	0	0	0
Centaurea maculosa Italy	0	0	0	0	0	0
Acroptilon repens Italy	0	0	0	0	0	0
Daucus carota Italy	0	0	0	0	0	0
Asparagus officinalis USA	0	0	0	0	0	0
Beta vulgaris (var. saccariphera) USA	0	0	0	0	0	0
Beta vulgaris (var. saccariphera) Italy	0	0	0	0	0	0
Beta vulgaris (var. alba) Italy	0	0	0	0	0	0
Beta vulgaris (var rubeus) Italy	0	0	0	0	0	0

Results and Discussions

Rearing: Results obtained from the P. inspersa rearing were successful with a total of 160 adults emerging. Emergence started at the end of July and continued through August, peaking in the middle of August with 90% of the larvae producing adults.

Males and females emerged simultaneously throughout the emergence period, with a sex ratio of 1♂: 1.05♀. Most of the adults emerged were used for "egg production". The soil type, and moderate watering are the two important factors in the successful rearing of P. inspersa from larvae to adult. In three years of P. inspersa rearings Rome laboratory has shown that sandy soil which retains low moisture should be used and extremely limited watering of the roots to avoid high soil moisture which can compromise larval development.

Egg Production: Two thousand twenty eggs were produced in the laboratory rearing program. Most of the eggs (80%) were found laid on leaves of the potted C. diffusa and the rest (30%) were laid on the upper part of the cages. During the month of August a total of 1560 eggs of P. inspersa were sent to Dr. S. Rosenthal, Albany, California for their testing. The remaining eggs (460) were kept in the Rome Laboratory and produced 270 larvae (59% viability) which were used to complete our testing. We have noted that the eggs of P. inspersa do not need additional water for hatching. Being dry, the egg chorion is brittle which facilitates the eclosion of the larva. If the egg chorion has absorbed water it becomes leathery and the first instar larva is not able to break the chorion so it remains trapped inside. Keeping the eggs dry during the pre-eclosion period is extremely important to producing a good hatch with the Pterolonche collected from Greece where the summers are hot and dry when Pterolonche eggs normally hatch in the field.

Adult Oviposition Behavior: Only one pair of five in the test laid eggs, so the results obtained from this trial should be considered tentative. The test will be repeated in 1985. At present, it seems that the female of *P. inspersa* prefers to lay her eggs around the midnight; with only a few eggs being laid during early morning and afternoon, leading us to hypothesize that *P. inspersa* is a nocturnal insect.

Conclusions

Based on three years of Laboratory and field investigations, *P. inspersa* clearly demonstrated that it feeds exclusively on diffuse knapweed including the North American ecotypes. Neither artichoke or safflower, the closest relatives of *Centaurea*, nor any of the other crop plants tested possessed the necessary combinations of chemical, structural, phenological and biological characteristics to sustain larval development of *P. inspersa*.

The *P. inspersa* larva causes remarkable damage to the root of its host, *Centaurea diffusa*, during its development. On medium size roots (5-10 mm diameter) larva produced irregular tunnels in the exoderm, cambium, and vascular system, reducing the storage capacity of the root and making them susceptible to the attack of fungi. Plants which survived the attack of *P. inspersa*, produced smaller and shorter flowering shoots which in turn produced a smaller number of flower heads than the shoots of unattacked plants. Investigations carried out in Italy and Greece indicated that *P. inspersa* is a suitable candidate agent for biocontrol of diffuse knapweed, and its release in the United States is recommended.

MUSK THISTLE

Rizza and Stazi

Cheilosia corydon (Diptera: Syrphidae)

In 1984, work on the phytophagous syrphid *Cheilosia corydon* (Harris) (= *grossa* Fallen) was concentrated in two different areas.

One objective was to supply enough additional information to the Working Group on Biological Control of Weeds to permit them to make a decision on the introduction of the insect for the biological control of musk thistle.

Our second objective was to study the biology of *C. corydon* in captivity.

The major objections to the release of the fly in the US were:

1. An insufficient number of plants of the California native *Cirsium crassicaule* were exposed in the first field trial.
2. Lack of data showing acceptance of US varieties of musk thistle in the field.
3. US varieties of artichoke were not tested in the field.
4. Confirmation of the identity of the *Cheilosia* material which was determined by the French taxonomist, Lyon.
5. The existance of several literature records, including Zwolfer's collections of *Cheilosia* spp. larvae on various *Cirsium* species.

All these problem areas pointed out by the Working Group have been resolved.

1. Fifteen more C. crassicaule plants were exposed to a heavy field population of the flies in a randomized field trial and oviposition occurred only on the control plants.

2. Several musk thistle taxa from the U.S., ie., Carduus nutans, Carduus thoermeri, and plumeless thistle (Carduus acanthoides) were all grown from seed obtained from Dr. M.K. McCarty's Carduus thistle garden at Lincoln, Nebraska.

These plants were exposed to a high population of Cheilosia flies in the field and were used in first instar larval survival trials in the laboratory. They were all accepted for oviposition in the field and first instar larvae transferred to them in the laboratory trials were able to complete their development. The annual Carduus pycnocephalus was included in the test but the plants were all infected with mildew so they were not in good enough condition to be considered valid test plants.

Also, a US variety of globe artichoke was tested both in the laboratory and in the field. The plants were not accepted for oviposition by the flies and first instar larvae transferred to them failed to survive, so US artichokes can be considered as unsuitable as host plants.

3. The Cheilosia flies examined by Dr. Lyon at INRA, Antibes, France were also examined by Dr. Chris Thompson USDA ARS SEL. Dr. Lyon's identifications were confirmed as being correct.

4. The phytophagous syrphid larvae obtained from various Cirsium spp. by Dr. Helmut Zwolfer (at the University of Bayreuth) were also sent to Dr. Thompson and the identities were confirmed.

In addition to filling in most of the lacunae in the petition for introduction, we were able to complete the studies on the biology of the adult fly.

The study started with the October 1983 collection of 100 roots of musk thistle (from Sila in Calabria) which were infested with mature larvae of _C. corydon_.

Three days after collection the roots were re-planted in the soil at Castel Porziano, our research site near Rome. The roots were divided into 2 groups of 50 each and covered by plastic screen cages 80 x 80 x 80 cm.

These smaller cages were within a larger 4 x 4 x 2 m saran cloth cage which would be used when the adults emerged. During a periodic check, on February 14, 74o and 55^{o} adult _Cheilosia_ had emerged in the small cages. At that time we also collected 140 unemerged puparia by digging in the soil within the small cages. All the puparia collected were in a vertical position with the anterior portion at the soil surface, exposed and not covered by soil, in such a way that when the fly was ready to emerge, it had only to break open the puparial cap and it was free to come out. From this finding, it is clear that when the mature larvae are ready to pupate they leave the plant and migrate to this position at the soil surface, so the teneral adult can emerge without obstacles. In order to slow down the eclosion and have control of the emergence of the flies, we put the 140 field collected puparia in a glass jar with fine sandy soil in the same position in which they were originally found and kept them in the refrigerator at 4^{o}C

In the large cage we tried to create the most natural habitat possible. The spontaneous flora like _Asphodelus_ and native grasses growing in the cage were left undisturbed and we added rosettes of _Carduus macrocephalus_ which were growing in the vicinity. In order to furnish the flies with pollen and nectar we added spontaneous species of _Calendula_ and _Bellis_ which were flowering at that time at Castel

Porziano and transplanted, into the large cage, small tree of *Mimosa* (*Acacia spectabilis*) which was in bloom.

The first group of adult flies 22^{0} and 22o was released in the field cage on February 17 and their behavior was observed daily until the last fly died.

By these observations we were able to learn when, where and how the adults mate, when and where the females oviposit and how the female searches to find the correct niche for oviposition. We also learned when and how the flies feed, and at what times they are active and quiescent. We also know the percent of puparia parasitized and the name of the parasite. Most importantly, we were able to obtain fertile eggs. Sixty nine percent of the eggs layed on *Carduus* in the cage were fertile.

These findings were confirmed when a second group of flies (10o and 20^{0}) was released in the cage on March 8. These flies all emerged from puparia removed from the refrigerator the previous day. Fifty two percent of the eggs from this group collected from musk thistle in the cage were fertile.

One testing artifact that came to light during this study of the biology and behavior of the adult fly is that in a cage, they will oviposit on plants other than the *Carduus* host (eggs were found on *Calendula*).

This points up the problem, that if the insect is introduced into quarantine and tested with a variety of plants in a space much more limited than the 32 m^3 field cage, it is predictable that they will get rid of their eggs on other than the host plant, thus confounding the specificity of this fly with unrealistic oviposition data.

We feel that the field tests and the larval survival trials give a realistic appraisal of the specificity of this fly and its preference for musk thistle.

Greek Laboratory

CENTAUREA SOLSTITIALIS (Yellow starthistle)

Rouhollah Sobhian

1. Remarks on Host Plant - Phenology (Sobhian)

Centaurea solstitialis (YST) in all stages (seedling, rosette, bolting flowering and senescing) were found in Kozani, Greece in September and October 1983. Several of these plants, which had flowered in 1983, were observed again in spring 1984, thus they had continued to grow, suggesting that the plant found in Kozani is biennial.

In October 1984 only seedlings and senescing plants could be found in the same area. The biennial phenology may occur if rain falls in late-summer or early-fall and triggers seed germination. Sufficient moisture would allow the seedlings to continue to grow. Perhaps this happened in 1983. But in 1984, which was very dry in late-summer and fall, seed germination may not have occurred, with the result that the plant became more of an annual.

Another interesting observation was made at the University farm in Thessaloniki where, in mid-August, water from a broken pipe flooded an adjacent piece of land. Small budding and flowering YST plants were found in the previously flooded area on October 4 and 5. This was the first time in 4 years of observation that budding or flowering plants had been observed in the Thessaloniki area in October. This suggests that water was a factor behind this growth pattern. Most probably these plants will continue to grow in spring 1985. Part of an experimental plot where YST was planted in spring 1984 was also flooded but the experimental plants senesced in October.

This supports statements in the literature about _C. solstitialis_ being an annual or biennial plant. However, as a rule, plants flowering in spring will be senescing in the fall.

2. _Urophora sirunaseva_ (Sobhian, Pittara)

About 10,000 flowerheads were collected between July 5 and 15 from Greek YST in Thermi. These were some of the first of the season since only 5-10% of the flowerheads were mature on anyone YST plant. This collection produced 43 females and 41 male flies, which were air freighted to Albany, California for release.

Twenty locations around Thermi were surveyed in October 1984 in an attempt to locate a source of _U. sirunaseva_ so large number of galls could be collected later for planned 1985 releases in California and Idaho. The infestations were very low; 0% in 4 samples, 1% in 5 samples, 7% and 6.5% in separate samples, and 2-5% in the rest of the samples (sample size was 100-200 heads per sample). YST patches are small in the Thermi area, so there probably would not be enough flowerheads to dissect for a sufficient number of Sirunaseva larvae for release in the US. At Thermi, only 100 _U. sirunaseva_ galls were collected for the 1985 releases in California. Arrangements were made to collect material during winter 1984 for 1985 releases in Idaho.

Eighty-seven adults emerged between May 15 and June 11, 1984 from 900 heads collected in the seed formation stage on September 7, 1983 at Kozani. The infestation was much lower at Kozani in 1984 as only 100 galls were found (n = 7000 flowerheads) and these were shipped to the USDA Albany Lab for isoenzyme studies. Another sample of over 100 galls was collected on Crete and sent to Albany for isoenzyme studies.

When the host plants were identified they were found to be Centaurea idaea instead of C. solstitialis.

3. Bangasternus orientalis Capiomont (Sobhian, Maddox, Pittara)

A field experiment was designed by Mr. D. Maddox, USDA Albany, California to determine the host specifficity of B. orientalis. Don Maddox participated in the experiment in Thermi from May 15 to June 16 and in beetle collections and releases in the plots, as well as in the first three data collections.

At the same time B. orientalis was released we also released the congener Bangasternus provincialis Fairmaire, a candidate for the biological control of Centaurea diffusa. The C. diffusa project report provides details about B. provincialis.

Procedure: A randomized complete block (RCB), consisting of 8 treatments (plant species or cultivars) replicated 8 times, (total of 64 blocks) was established at the University farm in Thessaloniki. The size of each block was 2 x 2 m and contained 3 individual plants grown in a row in the middle (see block design); 50 cm separated plants in a block.

The plant species and cultivars were selected on the basis of (a) botanical relationships, (b) host plant affinity, (c) economic importance and (d) native N. American plant considerations.

The test plants (treatments A through H) were:

A. Cirsium candelabrum: rosettes were collected along the road between Veria and Kozani on November 3, 1983 and were planted in appropriate blocks on November 4, 1983.

B. Carthamus tinctorius: safflower (cultivar US Hartman): seeds provided by D. Maddox were planted March 6, 1984 in "Jiffy sets" (a prepared peat moss planting medium) and transplanted in appropriate blocks on April 1, 1984.

C. Cynara scolymus: artichoke (US cultivar "Globe"): sent from California as small daughter plants were placed in the appropriate blocks on October 4, 1983.

D. Carthamus lanatus: seeds were collected in Thermi and planted in "Jiffy sets" on September 21, 1983 and kept outside on a balcony. No germination had occurred by October 6, so the seed coats of some of the pappus and non-pappus bearing seeds in the "Jiffy sets" were removed, the seed coat scarified and then replanted on October 18. Only two seedlings had appeared from the pappus bearing group by November 3, 1983 so the "Jiffy sets" containing the remainder of the seeds were moved to the field and planted in the ground to give them a chance to germinate during the winter and spring months. No more germination had occurred by March, 1984 but luckily one of the seedlings from October 1983 had grown to a small rosette and this made it possible to identify C. lanatus rosettes in the fields around Thermi. C. lanatus and C. dentatus usually occur together around Thermi and it is impossible to separate the rosettes of these two species. C. lanatus rosettes were collected on March 27, 1984 from a field where it was known that only this Carthamus species had grown in previous years.

E. Centaurea solstitialis, YST: field collected rosettes from Thermi were transplanted into the plot on March 15, 1984.

F. Centaurea calcitrapa: the plant is described as biennial so field collected rosettes from Ag. Anastasia (near Thermi) were transplanted into the plot on October 29, 1983.

H. Centaurea cyanus: field collected seeds from Kozani were planted in "Jiffy sets" on September 21, 1983, and germination started after 4 days, but it was very irregular. However, October 6 there were some good rosettes and seedlings with 4-5 leaves. On October 29, three vigorously growing rosettes were transplanted into each block designated to receive this treatment. Some of the plants were eaten by moles so it was necessary to replace them with small plants collected in Kozani on May 21.

The plot was weeded as needed to enable the test plants to grow unimpeded. However, not all of the weeds were removed at each weeding as it was decided to leave some resting and hiding places for insects. The test plants were generally in very good condition; a few were eaten by moles and most of the Cirsium did not flower, although they were in very good condition.

Adults of B. orientalis were collected from YST by the end of May in Kozani and by the first week of June in Thermi. B. provincialis were collected by early-June from C. diffusa plants in Thermi.

The adults were sexed and labeled with pink nail polish, as follows:

B. orientalis female:	One point on each elytra.
B. orientalis male:	One point on the right elytra.
B. provincialis female:	One point on the pronotum.
B. provincialis male:	One point on the left elytra.

In order to see if labeling with pink nail-polish would alter the mating behavior of Bangasternus spp., the following cage experiments were done:

Experiment I: Twelve females were placed in a refrigerator for 1/4 hour, after which half were marked with the pink nail polish.

One labeled and one unlabeled female were placed in a petri dish containing a male and fresh branch tips of the appropriate host plant (6 replicates). The beetles were observed for 4 hours. When a copulation ended within the 4 hour observation time, the male was replaced with anothe•.

The first copulation occurred after one hour and it involved an unlabeled female. Eleven copulations were recorded between.

Table 1. Randomized complete block design (3 plants per block) for Bangasternus spp. field experiment, Thermi, Greece, 1984 See Text and Table 2 for listing of treatment according to letter.

A	C	A	D	F	E	F	D
G	G	F	E	D	D	A	E
C	D	G	B	B	C	G	B
H	E	B	C	A	F	B	C
B	B	H	G	C	G	H	G
E	H	C	A	G	B	C	H
D	F	E	H	H	H	E	A
F	A	D	F	E	A	D	F

males and unlabeled females during the experiment. Males were not observed to copulate with labelled females.

Experiment II: Only labeled females were offered to males (1 pair/petri dish, 5 replicates and one control) in this experiment. Again, the pairs were observed for 4 hours. After 1.15 hours the first copulation was observed, which lasted for 1.5 hours. In the second replicate, copulation started after 2 hours and continued until the end of the experiment. In replicate 3, copulation started after 3 hours and continued to the end of the experiment.

No copulation was observed in replicate 4. In replicate 5, copulation started after 2.40 hours and continued to the end of the experiment. In the control, copulation started after 3.25 hours and continued to the end of the observation period.

In the case of *B. provincialis*, 5 labeled and 5 unlabeled females were individually paired in petri dishes with a male (the same nail polish was used to label the beetles). Copulation was not observed within a 3 hour period, however, males were observed copulating with labeled females released in the field (see field data tables).

Two pairs of each *Bangasternus* species were released in the center of each block on June 7 and the first field observation was made on June 8. Subsequent observations were made on June 11 and 14 with the final observation requiring 2 days, occurring on June 28 and 29.

At each observation, all the test plants in the test were checked and numbers of males, females and mating pairs, (labeled and unlabeled) that were observed were recorded. Special attention was paid to the presence or absence of *Bangasternus* spp. eggs on all plants and the eggs present, if any, were recorded. The entire data set was summarized in 32 table however, only pertinent aspects are summarized for this report.

Table 2 summarizes the data collected in the 4 observations for B. orientalis.

B. orientalis adults, especially males, were occasionally observed on Centaurea diffusa, but no eggs of this species were found on this plant. A few eggs were found on C. calcitrapa but no adults were observed on this plant during the observation periods. B. orientalis adults or eggs were not found on any other test plant.

Table 2. Number of female, male, mating pairs and unlabeled (UL) B. orientalis adults, and number of eggs recorded on each plant species during 4 observations.

Test plant species	No. of Bangasternus orientalis: Females	Males	Mating pairs	Unlabeled Adults	Eggs
YST (E)1/	73 (23,21,22,7)2/	49 (18,13,13,5)	48 (11,20,16,1)	3 (0,0,2,1)	3574 (80,566,1040,1888)
Centaurea diffusa(F)	1 (1,0,0,0)	6 (4,1,1,0)	0	0	0
C. calcitrapa (G)	0	0	0	0	17 (11,1,0,5)3/
Cirsium candelabrum (A)	0	0	0	0	0
Safflower (B)	0	0	0	0	0
Artichoke (C)	0	0	0	0	0
Carthamus lanatus(S)	0	0	0	0	0
Centaurea cyanus(H)	0	0	0	0	0

1/() Letters in parentheses refer to treatment as shown in Table 1.

2/ Numbers in parentheses represent data obtained in 1st, 2nd, 3rd and 4th observation periods.

3/ Dust infestation interfered with an exact egg counting.

Sampling:

1) Random sampling: On August 15, flowerheads of each plant species in each replicate were collected at random and checked for Bangasternus spp. eggs. The heads of artichoke and Carthamus spp. were placed in gauze bags. Samples, of the other plant species, were placed in plastic containers with holes to provide aeration for rearing out adults.

2) Egg sampling: Since egg and larval mortality had been very high in previous years, it was not expected that many adults would emerge from the samples. Thus, in order to try and rear-out larger numbers of adults, all YST and C. diffusa flowerheads or branch tips infested with B. orientalis and all (YST) or B. provincialis (C. diffusa) eggs were collected from August 16 - 30 and placed in gauze bags (one/replicate). In the case of C. calcitrapa, on which a few eggs of B. orientalis and B. provincialis were found, all of the flowerheads, about 7.8 kg, were collected (about 39,000 heads). It was necessary to do this because a heavy rust infestation which left dark pustules on the leaves and stems which contained most of the eggs made it extremely difficult to select those flowerheads with Bangasternus eggs.

At the end of September, when no more adults were expected to emerge, the random samples were checked and adults found in the containers were pinned for identification. All Bangasternus spp. eggs found on the flowerheads were checked to see if they had hatched. Those flowerheads where eggs had hatched were dissected to see if larvae had grown and to recover any adults still in the flowerheads.

Table 3 shows the results from the random samples. Of the 183 eggs found on the 8 YST plants, only 20 of them hatched and only one larva developed to an adult. The 3 eggs found on C. calcitrapa did not hatch. No eggs were found on other test plants (Table 3).

Table 3. Number of *B. orientalis* eggs counted on flowerheads or branch tips of each test plant species.

	Test Plants							
	YST	*Centaurea diffusa*	*Centaurea calcitrapa*	*Centaurea cyanus*	*Carthamus lanatus*	*Carthamus tinctorius*	*Cynara scolymus*	*Cirsium* candelabrum*
No. Flowerheads or Branch Tips Inspected	2800	800	800	741	800	800	123	111
No. of eggs	183	0	3	0	0	0	0	0

All of the "egg samples" were checked for adult emergence by early-October. Only 7 adults emerged from 3045 YST flowerheads (from 8 replicates) that had at least one B. orientalis egg.

In order to get more information about egg and larval mortality and the distribution of eggs on branch tips with a flowerhead, all YST flowerheads in replicates 1-3 of the field test were examined microscopically for the presence of eggs and then dissected to see if unemerged adults were present in the flowerheads.

Table 4. Data on *Bangasternus orientalis* mortality (egg-adult), and distribution of eggs on branch tips of *Centaurea solstitialis*

Replicate	No.of eggs found	No.of eggs hatched	Branch tips with 2 eggs	Branch tips with 3 eggs	Adults found in heads2/	Adults emerged in bags	Additional Remarks
1	655	146	49	6	3	2	Three eggs were found on 1 leaf
2	477	54	27	5	1	0	
3	219	27	221/	2	0	0	

1/ 3 eggs were found on 1 leaf.
2/ Adults found in heads were dead.

Oogenesis Studies

A preliminary study showed that B. orientalis females with developed ovaries will reabsorb their eggs if forced to feed on safflower in the laboratory, so we hypothesized that females would not develop eggs if they were forced to feed exclusively on safflower prior to ovarial development. An experiment and an observation were conducted to test this hypothesis.

Experiment I: Four field collected females were caged on safflower and YST bouquets (one per cage) on May 28. Cages were examined and plant bouquets were replaced with fresh material on June 4, 7. The results are shown below.

Date	No. Eggs Laid		No. Dead Females	
	Safflower	YST	Safflower	YST
June 4	0	80	0	0
June 7	0	24	2	0
June 11	0	93	2	0

All the females feeding on safflower died within 15 days after the experiment was commenced and none of the control insects on YST died. The dead females were dissected and no eggs were found in the ovaries of them, however one had 2 atrophied eggs. The four dead females on safflower also had empty digestive tracts.

In this observation 8 females were individually caged with safflower on June 19. The safflower bouquets were replaced every 3-4 days. The beetles were dissected at the time they died, which was between June 22 and 29. Two of the females had one atrophied egg, while the other six had no eggs. Female digestive tracts were empty

Cross mating between *B. orientalis* and *B. planifrons*

Beetles for this study were collected from their respective host plant (YST or *C. dentatus*) in Thermi and checked under a microscope to confirm their identity.

Experiment I: One *B. orientalis* female and one *B. planifrons* male were placed in a petri dish with samples of their host plants (3 replicates). One *B. orientalis* male and one *B. planifrons* female were set up the same way (3 replicates). The laboratory experiment was started on June 26 and repeated on June 27. The petri dishes were placed randomly on a table with a white background so the beetles would receive maximum light from a large window. After a 4-hr observation period on June 26 the petri dishes with beetles were placed in a refrigerator overnight. The test was repeated on June 27, following the same procedures except the observation period was reduced to 3-hrs. As a control, 3 *B. orientalis* males and females were paired in separate petri dishes with YST for food and 3 *B. planifrons* males and females were paired in separate petri dishes with *Carthamus dentatus* as food.

Table 6 Results of cross mating study between B. *orientalis* and B. *planifrons*

Replicate	Insect species and sex	Plants offered	Mating (+ or -)1/ June 26 (4 hr obs)	Mating (+ or -)1/ June 27 (3 hr obs)
	TEST			
1	B. *orientalis* male x B. *planifrons* female	YST, *Carthamus* 2/	-	+
2	" "	" "	+	+
3	" "	" "	-	-
1	B. *orientalis* female x B. *planifrons* male	" "	-	-
2	" "	" "	+	+
3	" "	" "	-	-
	CONTROL			
1	B. *orientalis* male x female B. *orientalis*	YST	+	+
2	"	"	+	+
3	"	"	+	+
1	B. *planifrons* male x female B. *planifrons*	*Carthamus*	+	-
2	"	"	+	-
3	"	"	+	+

1/ + = mating observed

2/ *Carthamus tinctorius* (safflower) was used.

Table 6 shows that mating occurred in all of the controls on June 26, and in 4 of the controls on June 27. Cross mating was observed between 2 pairs on June 26 and 3 pairs on June 27.

Experiment II: This small test was run by pairing one *B. orientalis* male with a *B. orientalis* female and one with a *B. planifrons* female (2 replicates). Also, one *B. planifrons* male was caged with a *B. orientalis* female and a *B. planifrons* female. A fourth petri dish contained a pair of each species. In order to avoid labeling the insects, large *B. planifrons* females and small *B. orientalis* females were used in the experiment so each species could be readily distinguished. Fresh food of both species was provided during the course of the experiment and petri dishes were randomly placed on a white background as described for the other experiment. The experiment started on July 9; it was repeated on July 11 and 12. Beetles were kept in a refrigerator on July 11 and overnight between observations.

In two instances, a *B. orientalis* male, after copulating with a *B. orientalis* female, copulated with a *B. planifrons* female.

These observations suggest that cross-mating can take place but it still has to be demonstrated that a female mated with a congeneric male is capable of developing fertile eggs, and if so, producing a F 1's without reduced fitness.

Table 7 shows the results.

Table 7. Cross-mating between _B. orientalis_ and _B. planifrons_ in a 2-choice test.

Repl.	Insect species and sex	Mating at 1st obs.	Mating at 2nd obs.	Mating at 3rd obs.
1	_B.orientalis_ ♂ x _B.orientalis_ ♀ _B.planifrons_ ♀	_B.orientalis_ ♂ x ♀	_B.orientalis_ ♂ x ♀ _B.orientalis_ ♂ x _B.planifrons_ ♀	_B.orientalis_ ♂ x ♀ _B.orientalis_ ♂ x _B.planifrons_ ♀
2	_B.orientalis_ ♂ x _B.orientalis_ ♀ _B.planifrons_ ♀	_B.orientalis_ ♂ x ♀	_B.orientalis_ ♂ x ♀	_B.orientalis_ ♂ x ♀
1	_B.planifrons_ ♂ x _B.orientalis_ ♀ _B.planifrons_ ♀	_B.planifrons_ ♂ x ♀	_B.planifrons_ ♂ x ♀	-
1.	_B.orientalis_ ♂ x ♀ _B.planifrons_ ♂ x ♀	-	_B.planifrons_* ♂ x ♀	

* _B. orientalis_ female was dead.

In preparation for possible 1985 studies, over 60 *B. planifrons* adults were dissected from *C. dentatus* flowerheads and placed in individual vials for overwintering. If time allows, surviving females will be mated with *B. orientalis* males to see if they will lay fertile eggs. It is very difficult, if not impossible, to field collect sufficient numbers of unmated females of *B. orientalis* to cross mate with *B. planifrons* males.

Distribution of Eggs on YST Patches in Thermi

A three hour survey on foot in each of the four cardinal directions from Thermi was made in order to collect data on egg distribution of *B. orientalis*. YST patches found in each direction were categorized as: 1) large patches (over 100 plants); 2) medium size patches (about 50 plants); and 3) small patches (up to 10 plants). Flowerheads (n = 50) were randomly collected from each YST patch and checked for the presence of *B. orientalis* eggs. A total of 32 small, 16 medium size and 14 large patches were found. The results show (Table 8) that the distribution of eggs on small patches was very irregular. The maximum number of eggs on 50 flowerheads (18 and 19) were found on small patches. Inspection of Table 8 also shows that eggs were found in all of the large patches and all but 2 of the medium size patches. Eggs were not found in 50% of the small patches. However when the mean number of egg per head is calculated for each category of infestation we found the small patches had only 0.058 eggs/head while the midium sized patches produced 0.105 eggs/head and the large patches 0.125 eggs per head, or twice the density found in the small plots. With this as a guide it seems that the most productive searches for eggs would be in the large patches.

Table 8 Number of *B. orientalis* eggs found on 50 flower heads examined from large, medium and small YST patches around Thermi, October 1984.

No. patches Observed	North			East			South			West		
	Large	Medium	Small	Large	Medium	Small	Large	Medium	Small	Large	Medium	Small
1	4	6	4	4	11	0	7	10	0	1	1	1
2	6	4	19	1	0	0	11	6	2	17	9	0
3	4	12	2		3	4	11	9	14	4	0	0
4	8		3		2	0	16	1	0		4	4
5	1		0			1		6	2			1
6			0			0			0			0
7			0			0			0			4
8									7			18
9									3			
10									0			

Additional Information on the Larval Behavior of *B. orientalis*.

Eggs are very often deposited on branch tips which do not develop a flowerhead or the bud dries-up before a flowerhead develops. In the past few years, many branch tips have been examined in order to find out the fate of a *B. orientalis* egg when this situation occurs.

Branch tips (n = 138) with *B. orientalis* attacked eggs were field selected for their lack of a developed flowerhead and the presence of an adjacent side branch with a developed head. The branch tips were carefully dissected under stereomicroscope and the following observations were made:

116 eggs hatched; 22 eggs did not hatch

93 larvae no trace found

13 larvae were found dead in their stem

2 larvae were found dead in the undeveloped bud

2 larvae were found dead in the 2nd branch they tried to mine

2 larvae were found dead in the 2nd bud they reached

2 pupae were parasitized in the 2nd bud

1 larva was found dead in the 3rd branch it tried to mine

1 larva was found to have reached a 2nd bud only do discover it was dried out.

In one case, an adult was found in the second bud the larva had entered. This evidence, together with the 2 parasitized pupae, shows that a *B. orientalis* larva does have the potential of completing its development in a bud, after first encountering and leaving an unsuitable one.

Laboratory Host-Specificity Studies

About 250 *B. orientalis* adults were collected and mailed to Albany for further host specificity tests in quarantine.

3. Chaetorellia hexachaeta (Sobhian, Pittara)

According to the literature and personal records of the host plants, this fly is restricted to a few Centaurea species. However, bouquets of buds of Cirsium, Zinnia and Carlina have served as oviposition sites for caged adults (for details see 1983 Rome Annual Report). One research objective for 1984 was to measure larval survival on those plants which were acceptable oviposition hosts for caged female flies.

Methods

Two methods were employed to measure larval survival on various plants after an initial attempt showed that it would be difficult, if not impossible, to transfer eggs from YST buds to buds of other plants without injuring the egg or placing it incorrectly on the flowerhead thus killing the unhatched larvae by dessication. With the first method, females were allowed to oviposit on Cirsium creticum and YST. When the heads were dissected 3-4 days post-oviposition, a few living larvae were found in 3 heads of C. creticum (Table 9). These neonate larvae were "black-heads", probably caused by undigested food in the anterior digestive tract, and appeared to be very weak and only nibbled a little before dying. In contrast, 15 of 17 YST flowerheads in the control had living translucent larvae with visible mouth hooks and appeared to be healthy. The two YST heads without living larvae were unsuitable hosts because they were too dry (Table 9).

In the second method, eggs laid on YST buds were dissected out and placed on moist filter paper in petri dishes for hatching. Eggs were checked in the morning and late afternoon each day. Using a brush made

from a single eye lash, neonate larvae were placed directly on achenes or between the distal row of achenes and the bracts, where eggs are normally deposited.

In the second method, larvae less than 1 day old were transferred to field collected buds of C. vulgare, C. creticum, Zinnia elegans, and YST buds. One larva was transferred to each bud, except for the 5 Zinnia buds which received 2 larvae each.

Table 10 shows that 1st instar larvae transferred to the test and control plants remained alive only in the YST control. However, there was some evidence of feeding in C. creticum and Zinnia buds.

The collecting results are encouraging enough to conduct more in-depth host specificity studies in 1985. Since the bouquets do not last long enough it is planned to repeat the test next year transferring the larvae to buds on potted plants instead of bouquets.

Table 9 Larval survival test with *Chaetorellia hexachaeta*, Greece, 1984

Replicate	YST (Control)				*Cirsium crecticum* (Test)			
	Date of Oviposition	Date of Examination	Results[1/] No eggs-	Date of No.larvae	Date of Oviposition	Results Examination	No Eggs	No Larvae
1	Aug. 7	Aug. 11	5	2[1/]	Aug. 2	Aug. 7	7	0
2	" 22	" 28	8	3	Aug. 4	Aug. 8	1	0
3	" 22	" "	1	1	" "	" "	11	0
4	" "	" "	4	1	" "	" "	11	0
5	" 23	" 30	1	1	" "	" "	10	0
6	" "	" "	2	0[2/]	" "	" 9	3	0
7	" "	" "	1	0[2/]	" 6	" 10	2	1 dead with blackened head
8	" "	" "	2	1	" "	" "	7	5 dead
9	" "	" "	3	1	" 8	" 11	3	3, black heads
10	" "	" "	3	1	" "	" "	10	5, " "
11	" 24	" 31	2	2	" "	" 13	8	4 dead
12	" "	" "	2	1	" 10	" 23	4	4 "
13	" "	" "	4	1	" 19	" 24	8	0
14	" 25	Sept. 1	2	1	" "	" "	2	0
15	" "	" "	7	1	" "	" "	2	1 dead
16	" "	" "	4	1	" "	" "	2	2 dead
17	" 26	" 2	7	2	" "	" "	1	1 dead

L = living larva

2/= flower heads were decayed or too dry.

Table 10. Results of *Chaetorellia hexachaeta* First-Instar Larval Survival Test.[1/]

Plant species	No. Buds	No. Larvae	Living Larvae	Dead Larvae	Remarks
YST	20	20	14	0	The rest of the larvae were missing
Cirsium vulgare	22	22	0	1	3 larvae of a different fly species were found in 2 heads.
Cirsium creticum	20	20	0	3	Buds partly rotten. Dead larvae with blackened heads.
Zinnia elegans	26	31	0	1	Buds mainly rotten. Plant needs to be tested using potted plants.

1/ Buds were dissected 5-6 days after receiving larvae.

Effect of Oviposition Pressure on Oviposition Behavior

An experiment was conducted to determine if female flies, having limited opportunity to oviposit on their host plant would also oviposit on non-host plants.

Methods: (Group A) Two pairs of *C. hexachaeta* were caged in a 1 liter transparent plastic containers with YST buds for oviposition sites and (Group B) 2 pairs of flies were placed in identical cages without living plants as oviposition substrate. There were 5 replicates per group and cages were provided with water and food. Flies for this study emerged July 9-11; the experiment started on July 11. Males were replaced if they died during the experiment. Buds were dissected daily under stereomicroscope and eggs were counted and recorded.

Group A received 2 fresh Bu-3 YST buds daily for 6 days. Group B received nothing. After 6 days, when 45, 32, 68, 40, and 33 eggs had been laid on the YST buds by the five Group A replicates, *Zinnia elegans* buds (1 per cage) were offered to both groups for one day. The next day, Group A was again offered only YST buds, then only *Z. elegans* on day 3, then only YST, etc. etc. In contrast, Group B flies had no oviposition substrates on days YST buds were offered to Group A flies. After each group had been exposed for 7 times to *Zinnia* buds, *Cirsium creticum* buds were substituted on alternate days for a total of 4 times.

During the last part of the experiment, after some flies had died, buds of *Carlina corymbosa* were substituted (3 changes) for the *C. creticum* buds. The experiment was stopped on August 13, after all the females in group A had died.

Results: The results of the experiment are shown on Table 11. The number of eggs laid by Group B females under oviposition pressure was very much higher on the "non host plants" than Group A females. For example Group B females laid 59 eggs on *Zinnia* and 101 eggs on *C. creticum*, while group A females laid only 5 eggs on *Zinnia* and 12 eggs on *C. creticum*. Group B females laid 4 eggs and group A 0 eggs on *Carlina corymbosa*, but these results were obtained with a few old females and are of questionable significance.

The oviposition behavior and fecundity of individual females is quite different. For example, the number of eggs laid on *Zinnia* by the 5 group B females, was 1, 0, 35, 0, and 23. Longevity of the individual flies was also very variable.

Table 11 Results of Laboratory Study to Measure Effect of Oviposition Pressure on Ovipositional Behavior of Chaetorellia hexachaeta, Greece, 1984.

Oviposition Choices																	
	Zinnia elegans[1/] and YST[2/]								Cirsium criticum[3/] and YST					Carlina corymbosa[4/] and YST			
	Bouquet changes								Bouquet changes					Bouquet changes			
Group A. Replicate	1st	2nd	3rd	4th	5th	6th	7th	Results	1st	2nd	3rd	4th	Total	1st	2nd	3rd	Total
	Eg YST	Eg YST	Eg YST	Eg YST	Eg YST	Eg YST	Eg YST	Eg YST	Cr YST	Cr YST	Cr YST	Cr YST	Cr YST	Co YST	Co YST	Co YST	Co YST
	No. eggs laid								No. eggs laid					No. eggs laid			
1	0 (13)	0 (19)	0 (18)	0 (21)	0 (14)	0 (15)	0 (3)	0 (103)	0 (10)	3 (11)	0 (3)	7 (1)	10 (25)	0 (2)	0 (6)	- (5)	0 (13)
2	0 (8)	0 (14)	0 (20)	0 (7)	C (0)	0 (6)	0 (5)	0 (60)	0 (8)	0 (4)	0 (6)	0 (6)	0 (24)	0 (3)	- (1)	-	0 (4)
3	0 (15)	0 (12)	0 (23)	0 (20)	0 (10)	- (2)	-	0 (82)	-	-	-	-	-	-	-	-	-
4	0 (20)	0 (20)	3 (15)	0 (22)	0 (18)	0 (18)	0 (21)	3 (134)	2 (16)	0 (18)	0 (25)	0 (9)	2 (68)	0 (4)	0 (0)	-	0 (4)
5	0 (16)	0 (24)	0 (7)	0 (23)	0 (13)	0 (26)	2 (5)	2 (114)	0 (1)	0 (3)	0 (8)	-	0 (12)	-	-	-	-
	0 (72)	0 (89)	3 (83)	0 (93)	0 (55)	0 (67)	2 (34)	5 (493)	2 (35)	3 (36)	0 (42)	7 (16)	12 (129)	0 (9)	0 (7)	- (5)	0 (21)
Group B Replicate																	
	No eggs laid								No. eggs laid					No. eggs laid			
1	0	0	0	0	1	0	0	1	0	11	4	0	15	0	0	0	0
2	0	0	0	0	0	0	0	0	2	22	4	10	38	0	0	3	3
3	[illegible]	0	7	8	1	18	1	35	7	11	9	8	35	-	-	-	-
4	0	0	0	0	0	0	0	0	0	1	0	0	1	0	0	0	0
5	0	0	6	0	1	9	7	23	1	10	0	1	12	0	0	1	1
Total eggs laid	0	0	13	8	3	27	8	59	10	55	17	19	101	0	0	4	4

1/ Zinnia elegans = Eg

2/ Centaurea solstitialis = YST (control)

3/ Cirsium creticum = Cr

4/ Carlina corymbosa = Co

Bionomics and Behavior

1. Nine samples of 1000 seedheads each of YST were collected weekly from June 29 to August 13, 1983. The same plants were sampled on each date and only heads in the seed formation stage were collected, and all the heads collected were placed in gauze bags and stored out of doors. Adults emerged in July and August 1983 and in April and May 1984 from five of the six samples collected between July 13 and August 13, 1983. showing that the presence of adults of the first and second generation overlap in the field. In 1983, the last adults emerged on August 14 and 15 from samples collected on August 6 and 13. In 1984, the first adults emerged on April 25 and the last ones on May 15. The sex ratio was about 1:1.

2. In the laboratory, up to 11 eggs have been found under a single bract of a YST bud. However, examination of field collected flowerheads showed that mostly 1 egg was deposited on a bud, but occasionally two were noted.

Observation of ovipositing females in the laboratory revealed that they usually only lay one egg at a time but some have been observed to frequently lay many eggs under a single bract, one at a time.

3. Plot studies showed that females will readily oviposit on YST buds (Bu-3) that are cut and air dried. Also, females emerging from *Centaurea cyanus* will readily oviposit on YST buds.

4. It is possible for *C. hexachaeta* and *U. sirunaseva* to co-exist in the same flowerhead, because we found a few flowerheads that contained both a cocoon of *C. hexachaeta* and a gall of *U. sirunaseva*. Also, two *C. hexachaeta* larvae were occasionally found in one flowerhead. One would not expect to find many heads harboring both species in the field because of low infestation rates for both tephritids.

4. Eustenopus abbreviatus (Sobhian, Pittara)

The Eustenopus described in last year's annual report as villosus has since been identified as E. cf. abbreviatus by Dr. Whitehead (Research Entomologist, USDA Systematic Entomology Laboratory).

In 1981, one egg and one first instar larva of this weevil were transferred from YST buds to SF buds. There was a control consisting of three first instar larvae transferred from YST to YST buds (1/bud). After two days, the larvae were found feeding in the SF and YST buds. After another 5 days, two larvae were found dead in the two SF buds; however, larvae were still feeding in YST buds. It was decided to collect for data in 1984 to measure the ability of larvae to survive in various hosts under laboratory conditions.

A. Larval Survival Test

On July 2, 1984 4 eggs were transferred from YST buds to safflower buds and 4 eggs to YST buds (1 egg/bud). The buds had been removed from field plants and were kept as bouquets in a cage. The eggs were inserted into buds attempting to place them just as a female weevil would.

By July 11, 3 eggs had hatched in safflower and the larvae were feeding normally. On the same day these 3 larvae were transferred to fresh safflower buds (1/bud). Also, 3 of the 4 eggs in the YST buds hatched but one died because its bud had dried out, one was lost, and one was feeding normally, so it was transferred to a new YST bud. In the YST bud without a larva a Chaetorellia pupa was found, leading us to speculate that the Chaetorellia larva killed the Eustenopus larva.

On July 18, an examination of the YST and safflower buds was made

and only 2 larvae could be found and these were feeding in safflower; on the same date these larvae were transferred to safflower buds on potted plants. On August 2 one living Eustenopus pupa was found in a safflower bud. The pupa completed its development, metamorphosing to an adult by mid August, showing that one larva was able to develop to the adult stage when transferred from one safflower bud to another. The egg to pupa development time was about 4 weeks. The pupal stage lasted about 2 weeks.

B. Oogenesis Test

Eustenopus adults (n = 52) were collected in fall 1983 by dissecting them out of YST heads then held overwinter in a plastic container with plant debris in an unheated laboratory. At the end of February, 1984 the beetles were still in diapause but some of them were dead. Between April 23 and 25, 1984, when the first adults were observed moving around in the container (end of diapause), 20 unsexed adults were removed and half were caged with a bouquet of safflower and half with YST. On May 11, 9 more active unsexed adults were caged with a second safflower bouquet. The tenth adult was added to this last safflower bouquet on May 14. Bouquets were replaced with fresh ones about once a week. Adults that died on safflower were dissected to determine their sex and to examine egg development in the females. The results are summarized below:

Dissection Date	Males	Females	Developed Eggs
May 21	3	5	0
June 22	7	1	0
June 25	0	1	0
July 5	0	2	0
July 12	0	1	0
	10	10	0

In a control experiment with 10 insects on YST bouquets, laid 36 eggs. Two of the 10 insects were found to be females. The 10 females which died on safflower did not lay any eggs. It is concluded that females need to feed on YST to develop eggs.

However, some of the adults lived for 3 months on safflower. This observation plus the fact that one larva was reared from egg to adult on safflower lead me to conclude that *E. abbreviatus* is a potential pest of safflower and should not be considered as a biocontrol candidate for YST.

C. Oviposition Behavior

Females normally select mature flower buds for oviposition. The oviposition behavior of a female was observed for 2.30 hours in the laboratory. She spent 70 minutes making an oviposition hole in a YST bud with her mouth parts, after which she turned around, placed the tip of her abdomen into the hole for 10 minutes and laid an egg. She then covered the hole with a substance resembling feces. After 5 minutes she positioned herself using the tarsi of her hind legs for a few

minutes to press and compact the covering. A second female made an oviposition hole in the same bud but did not finish it within 2.30 hours of observation, but the next day a normal egg was found in the hole.

D. Bionomics and Behavior

1. When the females started to oviposit on YST bouquets, the bouquets were replaced daily and the number of eggs laid per day was recorded. The peak of oviposition was around mid July, when the two females laid up to 9 eggs/day and up to 5 eggs/bud (only 2 buds/day were offered). A total of 135 eggs were laid by the females from June 25 to August 13. The last female stopped laying eggs three days before she died.

2. Teneral and young adults are dark brown with one longitudinal white strip on each elytra, but as they age they become yellowish and the white bands on the elytra become less pronounced.

3. Two YST flowerheads, each infested with 2 Eustenopus eggs, one on August 2 and one on August 11, were examined several days later. One well developed larva was found in each head but in one, the head capsule was all that remained of the second larva. Perhaps larval cannibalism occurred?

4. Numerous field collected YST buds with B. orientalis eggs were offered as bouquets to ovipositing Eustenopus females to determine if they would oviposit in a bud previously selected by B. orientalis.

The buds were readily accepted for oviposition, but, because of high natural mortality in the Bangasternus eggs, only 3 eggs hatched with the corresponding larvae reaching the buds. Eight to 10 days after oviposition by Eustenopus only one living larva was found in each

bud, and one bud also contained a partially eaten weevil larva. We were unable to rear-out the living larvae to adults; however, based on many observations of larvae of this species, the larvae did not appear to be Bangasternus, so we assume that the surviving larvae were Eustenopus and that it is a more aggressive species.

5. Baseline Studies

A small field experiment was planned by Drs. S. Clement and R. Sobhian for studying the insect guild attacking YST from different areas, including the U.S., and grown together under uniform field conditions in Greece.

Seeds of YST from three U.S. locations (CA, WA, ID), plus Greek seeds, were planted in "Jiffy sets" on March 3, 1984 and kept in a greenhouse until rosettes were transplanted into a field plot at Thermi on April 14. These rosettes, along with field collected C. diffusa rosettes, were grown in a 5 x 5 Latin square configuration (2m between plants in a row and 2.3m between the rows). Since the YST plants were late in flowering and were very small, we were forced to abandon plans to collect all of the desired data. However, all mature seedheads of each YST ecotype were collected on October 17 and examined for seed feeding insects. The results are shown in Table 12.

The level of infestation by flowerhead insects was relatively high. The Idaho plants had the highest infestation, in respect to number of species and number of larvae/100 flowers. We were encouraged by the fact that the cynipid gall wasp infestation Isocolus sp. level was the highest so far observed in northern Greece. was on the Idaho plants.

Table 12 Insect guild in seedheads of various ecotypes of *Centaurea solstitialis*, experimental plot, Thermi, Greece 1984.

Insect	Stage or Form	YST Ecotype (Source of Seed)			
		Idaho	Washington	California	Greece
Bangasternus orientalis	eggs	0	0	1	0
Chaetorellia spp.	larvae, galls pupal exuvia	2(2heads)	5(3 heads)	4(4 heads)	0
Urophora sirunaseva	galls	10(6 heads)	0	0	0
Bruchidius sp.	adults or pupal exuviae and chamber	3(3 heads)	0	1	0
Metzneria sp.	larvae	5(5 heads)	1	0	0
Isocolus sp.	galls	60(17 heads) (mainly in seeds)	10(6 heads)	47(25 heads) (mainly in bracts)	11(5 heads)
Acanthiophilous sp.	exuviae	0	2(2 heads)	15(6 heads)	13(4 heads)
Larinus spp.	pupal exuviae	3(3 heads)	0	1	1
Lasioderma sp.	larvae	0	0	19(18 heads)	0
Total No. Seedheads collected:		89	32	123	44

6. Exploration

Surveys for additional natural enemies continued in 1984.

1. Surveys were conducted on Crete from September 9 to 17. The objective of the survey was to locate YST infestations that could be surveyed again in spring 1985 for rosette feeding insects. A student in the Faculty of Agriculture in Thessaloniki (Mr. S. Kashefi) provided information on location of YST infestations on Rodos and also some samples of YST flowerheads and infested roots. *Centaurea solstitialis* was not found in Crete, however a similar appearing perennial *Centaurea idaea* was found in abundance.

A cerambycid beetle was collected in the roots of plants resembling *Centaurea solstitialis* on the island of Rhodes in July. The roots were dissected on October 30, after they were kept for about 3 months in plastic containers containing moist sphagnum moss. Five adults and one larva were found in their mines. This beetle may not be an effective biocontrol agent because its larvae complete their development in dead roots during late-summer and fall. Specimens will be submitted for identification to the USDA Systematic Entomology Laboratory.

2. Surveys around Thermi resulted in the discovery of a cynipid wasp which galls the stems. The galls were collected from June 26-30 and some small wasps were reared out. These will be submitted for identification to the USDA Systematic Entomology Laboratory. It is not known if the reared material are cynipids or their parasitoids. This insect appears to be quite rare. Perhaps it is the same one found by Dr. S. Rosenthal during her 1984 survey of Turkey.

CENTAUREA DIFFUSA (Diffuse knapweed)

R. Sobhian

1. Remarks on the host plant

C. diffusa is known as a biennial weed, however there is not enough information available on the phenology of the plant to allow us to predict when it will be useable in host specificity tests. For example, when we needed *C. diffusa* rosettes for *Pterolonche* oviposition in August 1983, we did not know what time to plant seeds in order to have good rosettes in August. On another occasion we wanted to test the plant in a field experiment with *Bangasternus* spp. and we were facing a similar problem, that is we did not know if seeds planted in the fall 83, would provide us with flowering plants in June 1984.

To get a better understanding of the phenology of the plant three seed samples of Greek origin, were planted at different times as follows:

A) seeds were planted in September 23, 1983 in "Jiffy sets" (small peat pots) and the resulting rosettes were transplanted into a field at the Univeristy farm, Thessaloniki, on November 4, 1983 (30 small rosettes).

B) Seeds planted on March 4, 1984 in a wood flat were kept in the greenhouse until April 11 and 97 of the resulting rosettes were transplanted into the same field, as group A,.

C) The wood flat in which the seeds were planted on March 4 was kept in the greenhouse and watered after transplanting the small rosettes in April. Again numerous seeds germinated in the container and 56 of these small rosettes were transplanted near the other two groups in the field on May 16.

The majority of the plants which survived from group A were budding on June 8 and all of them flowered in the summer 1984.

All the plants in the three treatments were examined on Sept. 25, 1984, and their growth was recorded. All the plants surviving in group A flowered in the summer 1984, but only two were senescing in September. In group B, only half of the plants that survived were flowering and the rest were in the rosette stage. From the 39 plants surviving in group C, only 7 were flowering and the rest (32) remained as rosettes. The result of this experiment is shown on Table 1.

Table 1. 3 treatments (A, B, C) showing the phenology of *C. diffusa* in the first year.

Treatment	Sowing date	Date of transplanting	No. rosettes transplanted	No. plants found(9/25/84)	No.flowering plants(9/25/84)	No. senescing plants(9/25/84)
A	Sept.23/83	Nov. 4, 83	30	0	7	2
B	March 4, 84	Apr. 11, 84	97	39	31	0
C	March* 4, 84	May 16, 84	56	32	7	0

* late germination (see text). Germination started on April 18.

Many of the flowering plants in the three treatments started a secondary growth, which shows that they are going to continue to grow in 1985. It was also noticed that many of the *C. diffusa* rosettes, transplanted for the 1984 *Bangasternus* field experiment, started a secondary growth on October 1984.

The experiment shows that a high genetic variability exists among the wild population of *C. diffusa* in our area and provides us with the planting date information needed to have the plants in the correct stage for a field trial.

2. *Bangasternus provincialis* (Sobhian, Maddox, Pittara)

The host specificity of the diffuse knapweed seed head weevil *B. provincialis* was studied along with *B. orientalis* in a field experiment carried out in Thermi. The block design, test plants and procedure were the same as those described for *B. orientalis* in connection with YST project. For origin of material, labelling procedure, release, field observations and method of final evaluation, see yellow starthistle project - *B. orientalis*.

Results

The results of the 4 field observations and the collected data on the presence of adults and eggs on the test plants is shown in Table I. Adults were observed only on *C. diffusa*, their original host plant, *C. calcitrapa* and occasionally on YST. Eggs were found only on *C. diffusa* and *C. calcitrapa*. No eggs or adults were observed on any other test plant.

Table I: Number of ♀♀, ♂♂, mating pairs and unlabelled (UL) _B. provincialis_ adults and number of eggs registered by 4 observations on each plant species

Plant species	♀♀	♂♂	Copulating pairs	UL (Feral)	eggs
Centaurea diffusa	53 (14,18,18,3)*	71 (21,20,19,11)	20 (0,10,5,5)	36 (8,14,12,2)	443 (2,71,82,288)
Centaurea calcitrapa	21 (6,9,4,2)	18 (6,7,5,0)	3 (0,0,3,0)	6 (0,2,4,0)	58** (0,3,31,24)
Centaurea solstitialis	1 (1,0,0,0)	4 (2,0,2,0)	0	0	0
Cirsium	0	0	0	0	0
Carthamus tinctorius	0	0	0	0	0
Cynara scolymus	0	0	0	0	0
Carthamus lanatus	0	0	0	0	0
Centaurea cyanus	0	0	0	0	0

* Numbers in parentheses represent the number and origin of adults and eggs recorded during each observation from 1-4 on.

** Plants were infested with rust; egg counting nearly impossible by the 4th observation.

The rate of infestation of the seed heads of the field test plants with _B. provincialis_ eggs and the number of adults reared from up to 100 seed heads are shown on Table II. Here, again, eggs were found only on _C. diffusa_ and _C. calcitrapa_ and adults emerged only from _C. diffusa_.

All _C. diffusa_ seed heads infested with at least 1 _B. provincialis_ egg were collected from all replicates in the field trial and kept to rear out adults. Since the number of adults that emerged from the samples was very low the seed heads collected from replicates 1-3 were examined and the rate of egg and larval mortality was determined from the eggs found in the samples. Number of eggs/seed head or branch tips with a developed seed head also has been determined. The result of this examination is shown on Table III. Out of 433 eggs found in the three samples 211 hatched. All seedheads on which eggs had hatched were dissected. High egg parasitism and other lethal factors were involved in egg mortality. Only 2 adults hatched in the samples and 3 dead adults were found in the seedheads.

Table II Number of _B. provincialis_ eggs counted on 100 flower heads/plant species (except artichoke) and number of adults emerged from the samples.

Plant species	_Centaurea diffusa_	_Centaurea calcitrapa_	YST	_Centaurea cyanus_	_Carthamus lanatus_	_Carthamus tinctorius_	_Cynara** scolymus_	_Cirsium* candelabrum_
No. of eggs	38 (14)	9 (2)	0	0	0	0	0	0
No. of adults	2	0	0	0	0	0	0	0

* 7 of the replicates did not yield 100 heads.
** Because of plant phenology number of heads in all replicates was smaller than 100.
( ) Numbers in parenthesis show the number of eggs that hatched.

Table III The rate of *B. provincialis* mortality (egg-adult) in 3 replicates and distribution of eggs on branch tips.

Rep.	No. of eggs examined	No. of eggs hatched	Branch tips with 2 eggs	Branch tips with 3 eggs	Adults found in flowerheads	Adults emerged in bags.
1	116	69	1	0	2*	0
2	103	60	2	0	0	1
3	214	82	3	0	1*	1

* The adults were dead.

Conclusions and Comments

For the following reasons *B. provincialis* is strongly recommended as a candidate for biological control of *C. diffusa*:

1. No negative result on the host specificity of the species was obtained in our field experiment.

2. No economic plants are reported in the literature as hosts for *B. provincialis*. Zwolfer reared the species from *Centaurea paniculata* in southern France (C.I.B.C. report No. 1, 1965) and Hoffmann (1953) reared it from *Centaurea nigra*.

3. The species is not race specific, it also oviposits on *C. calcitrapa*.

4. In a sample of 10 seedheads, (in which one larva has been developed to adult or pupa) a 100% of seed consumption has been recorded.

5. A closely related species of the genus, *B. orientalis*, has been found specific enough to prepare a petition for its release on yellow starthistle.

6. The oviposition period is fairly long (June - September) so that it is synchronized with the main part of the flowering season of *C. diffusa*.

Additional information on bionomics and behavior

1. Hibernation: On March 4, 1984 two adults were found in a sample of *C. diffusa* seedheads collected on November 2, 1983 and kept in gauze bags in an unheated room to overwinter.

2. Many larvae failed to mine into the plant tissue. Very often dead larvae were found, which were partially in their egg caps, did a little feeding trying to mine into the plants but failed to do so.

Many larvae were found dead at the location where they have to mine from leaf petiole to the stems. Dead larvae were also found in their mines in the stems.

3. When eggs are laid on leaves the larvae mine directly from the egg caps to the leaves. The smaller larvae of B. provincialis and thicker leaves of C. diffusa make it possible to mine directly into the leaves. B. orientalis larvae eat a furrow along the YST leaves and mine to the leaf petioles.

4. Éggs were frequently laid on branch tips, which did not develop a flowerhead. Such larvae, like B. orientalis larvae, turn back and mine downward searching for the nearest side branch. Probably some of these larvae will succeed in finding another branch with a proper bud in which they can complete their development.

5. Adults were found on C. diffusa plants in Thermi as early as the third week of May (rare) and as late as September 16. In June large number of pairs and single adults (up to 25 individuals on one plant) were found in Thermi.

3. Pterolonche inspersa

Fertile eggs of this species were needed for host specificity tests in quarantine at Albany, CA. Since the number of eggs oviposited by the adults in Rome was not sufficient to complete the tests in Albany, we were notified by telephone from Rome, in mid-August, to collect a few thousands fertile eggs from C. diffusa rosettes in Greece. On August 16, 274 eggs were collected, and of these 146 eggs (53%) had hatched.

If the situation next year is like this year, it will be possible to collect a few thousand fertile eggs, just around Thermi, in early-August. As an interesting side light, we discovered that the

eggs were not laid only on rosettes, as expected, but also on flowering but still green C. diffusa plants.

4. Exploration for additional natural enemies

A trip was made to Crete to explore for additional natural enemies of Centaurea spp. Unfortunately not a single C. diffusa plant was found in the island of Crete. However, in the area near Thermi the following candidates were found attacking this plant species:

1. An eriophyd mite, most probably Aceria centaurea was found on C. diffusa rosettes on September 30 near Arnea, about 40 km. from Thermi. Infested rosettes were quite common and many were seriously damaged by the mite which might be a good candidate for biological control of C. diffusa. A sample has been collected and sent to Dr.ssa Castagnoli at the Istituto di Zoologia Agraria, Florence for identification.

2. A leaf mining larva was found on C. diffusa rosettes in September. Since many leaf miners are known to be specific and since the insect attacks the plant at a time when its rosettes are stressed for water this leaf miner may be a potential candidate for biological control of C. diffusa. Twelve specimens were reared out, pinned and sent for identification. Larval parasitism was high. The species may already be known to colleagues working with C. diffusa.

3. A small curculionid leaf miner has been found in Thermi in C. diffusa leaves, during the second half of August, and 2 specimens have been reared for identification. The infested leaflets are slightly swollen, so one is uncertain whether to categorize the larvae as leaf miners or gall makers. The larvae pupate in their mines

Preparation for 1985 season

Two C. diffusa seed samples, provided by the USDA Laboratory in Albany, CA. were sown in two pots on October 15. By October 30, 30 seedlings were counted from one sample but only 3 from the other. It is planned to conduct an experiment and find out whether B. provincialis will accept the resulting plants when they are mature next summer.

Miscellaneous

A. Euphorbia spp.

1. On April 7, one female emerged from 4 pupae of Simyra dentinosa reared from E. esula in 1983. This female has been dissected. Large numbers of eggs were found in her ovaries.

2. Seven Chamaesphecia sp. adults were reared from a few roots of E. seguieriana collected by Volvi lake near Thessaloniki. The infested roots were collected on April 7 and the adults emerged April-May. The specimens have been sent for identification.

Since the larval survival test of Chamaesphecia sp., attacking E. esula (sensu latu) in Hungary, has been negative on US leafy spurge, it is recommended to examine the larval survival of the species attacking E. seguieriana roots. A cerambicid beetle, Leptura sp., has been reared from E. seguieriana. Dr. Schroeder (C.I.B.C.) indicated the Leptura sp. he worked with is a general feeder. It is not sure that this is the same species with the same degree of host specificity of the one tested by Dr. Schroeder.

B. Galium sp.

Heavy gall formation was found on Galium sp., near Kozani on May 15. A cecidomyiid species was responsible for the damage. A sample of the larvae was collected for identification. Large number of swollen leaflets were clustered together at the branch tips of the infested plants making more or less spherical hard galls, preventing the plants from growing, flowering and seed formation.

C. Centaurea maculosa

Fifty seedheads of a sample collected at Panagia Soumela, on October 7 were dissected. Eight Urophora galls (probably U. quadrifasciata), one Chaetorellia larva (C. hexachaeta?), two Isocolus sp. galls and two pupal exuviae of a Trypetid fly were found in them.

D. Centaurea calcitrapa

A sample of seeds collected near Thermi were sown on September 23, 1983. About 30 rosettes resulting from the seeds were transplanted to a field at the Unversity farm, Thessaloniki on November 4, 1983. Nine plants survived, and flowered in summer 1984. On November 1, 1984 one of them had started a secondary growth, while the others were senescing.

A second sample, from the same source of seeds, was sown on March 1, 1984 and the resulting rosettes were transplanted in the same field at the University farm, on April 11. Eighteen rosettes were living on October 1984. None of them flowered in the 1984 season.

This plant is also known as a biennial weed but its phenology is quite different from C. diffusa, which also is described as biennial.

E. Two short seminars were given by Dr. Sobhian in Greek to two groups of about 90 students of the Faculty of Agriculture, Thessaloniki, explaining them some of the principles of biological control of weeds and its methods, the purpose of our survey in Greece and the YST project and its special problems in the USA. A small exhibition of the insects found on YST in Greece, with emphasis on the promising candidates, was arranged for the students, and the students were given a guided field tour to the field where they could see our experimental plot, which has been designed for testing the host specificity of _Bangasternus_ spp.

A brief explanation about the YST project and the running experiments was given by Dr. Sobhian in German to Prof. Zwolfer's students, from the University Bayreuth, Germany.

F. _Insects for identification_

The following 216 specimens were sent for identification in 1984:

a) from YST:

34 _Eustenopus_ sp. (reared from YST)

52 _B. orientalis_ (voucher specimens)

10 _Urophora_ sp. (reared from YST)

12 _Larinus_ sp. (2 different species) (collected on YST)

16 _Lixus_ sp. from Crete (dissected from roots)

2 _Lixus_ sp. from Kozani (coll. on) and 2 _Lixus_ sp. (feeding in stem galls (reared))

6 Cynipid (stem galls) (reared)

5 Cerambycidae from Rodos (reared)

b) from C. diffusa:

4 Eustenopus sp. (collected on)

51 B. provincialis (voucher specimens)

11 Diptera leaf miners (reared)

2 Curculionidae leaf miners (reared)

c) from E. seguieriana:

7 Chamaesphecia sp. (reared)

d) from Carthamus lanatus:

4 specimens of B. planifrons for confirmation (collected on)

VISITORS - GREECE

March 19-23	Dr. Stephen L. Clement, USDA, ARS, Rome Laboratory
April 7-9	Dr. Sara Rosenthal, USDA, ARS, Albany, CA.
April 18-22	Prof. H. Zwolfer, University Bayreuth, Germany
May 6-10	Mr. Ben Kopacz, Assistant to the Administrator for International Activities, USDA, Beltsville, MD.
May 15-27	Mr. Gaetano Campobasso, USDA, ARS, Rome Laboratory.
May 17-June 16	Mr. Don Maddox, USDA, ARS, Albany, CA.
May 23-June 2	Dr. Stephen L. Clement, USDA, ARS, Rome Laboratory
May 21	Dr. William Bruckart, USDA, Frederick, MD.
June 14-16	Dr. Schroeder and his assistant, C.I.B.C., Delemont, Switzerland
June 14-16	Prof. H. Zwolfer accompanied by Dr. P. Hartmann and a few students, University Bayreuth, Germany
June 14-16	Dr. R. Nowierski and his assistant, Montana State University, Bozeman, MT.
July 25	90 Students accompanied by their supervisor Dr. D. Stamopoulos from the Faculty of Agriculture, Thessaloniki.
July 29-Aug. 2	Dr. Stephen L. Clement, USDA, ARS, Rome Laboratory
July 29-Aug. 2	Dr. David Perkins, USDA, ARS, Parasite Laboratory, Paris, France.
Sept. 20	Mr. G. Shelden, Agricultural Counselor, American Embassy, Athens
Sept. 20	Dr. A. Trimis
Oct. 12-16	Mr. D. Coutinot, USDA, ARS, Parasite Laboratory, Paris, France.
Oct. 25	Prof. Tzanakakis and Prof. Katsoyannos, Unversity, Thessaloniki.

PUBLICATIONS

Pecora, P., and P.H. Dunn. 1984 Suggested weeds for biological control. Proc. EWRS 3rd Symp. on Weed Problems in the Mediterranean Area. pp. 373-380.

Rizza, A., and P. Pecora. 1984. Chrysolina gypsophilae (Coleoptera: Chrysomelidae), a potential biocontrol agent of dalmatian toadflax, Linaria dalmatica (Scrophulariaceae). Ann. Entomol. Soc. Am. 77: 182-187.

TRAVELS (Rome Lab.)

February 6-8	Clement and Cristofaro to Puglia to survey yellow starthistle.
February 15-17	Stazi to Rovereto to confere with Prof. Tamanini.
February 17-19	Laregina to Padova to visit the Floraculture Fair.
February 27-March 3	Dunn, Pecora, Clement, Campobasso to France to participate to the annual meeting with CIBC and CSIRO scientists.
March 13-14	Clement and Mimmocchi to Castel del Monte (Puglia) to establish yellow starthistle plots.
March 19-23	Clement to Greece to survey Yellow starthistle and coordinate research with Dr. Sobhian.
March 21-22	Pecora and Cristofaro to Pisa to collect *E. esula* plants.
April 2-6	Pecora to Portugal to present a paper at the 3rd Symposium on Weed Problems in the Mediterranean Area.
April 3-4	Cristofaro to Pisa to work on *Dasineura* plots.
April 9-14	Campobasso to Bari and Brindisi to collect *Cyphocleonus morbillosus* adults on *Centaurea solstitialis*.
April 9-10	Clement and Mimmocchi to Puglia to collect *Apion* on yellow starthistle.
April 11-12	Pecora to Pisa to organize the *Dasineura* field test.
April 16-18	Pecora and Cristofaro to Pisa to organize the field test with *D. capsulae*.
April 26-28	Campobasso to Salerno and Bari to collect *Centaurea alba*, a host plant of *Cyphocleonus* spp.
May 3-4	Cristofaro and Stazi to Pisa to collect *Neoplinthus* larvae, *Bayeria* galls and check *Dasineura* plots.
May 7-9	Clement and Mimmocchi to Puglia to collect *Apion* on yellow starthistle.

May 14-28	Campobasso to Greece to collect *Sphenoptera yugoslavica* on *Centaurea diffusa*, *Bangasternus orientalis* on *Centaurea solstitialis*, and *Pterolonche inspersa* on *Centaurea diffusa*.
May 16-18	Murano to Campania to collect *Cyphocleonus morbillosus* F. a root feeder of *Centaurea solstitialis*.
May 17-18	Pecora and Cristofaro to Pisa to collect *Bayeria* galls.
May 22-24	Pecora to Bari to confere with Prof. Solinas.
May 23-June 9	Clement to Greece to collect *Apion* on yellow starthistle.
May 30-31	Pecora to Pisa to collect *Bayeria* galls.
June 6-8	Pecora to Pisa to collect *Dasineura* galls.
June 9-25	Rizza and Stazi to Austria and Hungary to collect *Oberea*, *Dicranocephalus*, *Bayeria*, *Dasineura* and *Euphorbia* roots.
June 13-19	Dunn and Clement to Puglia to collect on yellow star.
June 19-24	Pecora to Pisa and Ferrara to collect *Aphthona flava* on *E. esula* and *Lobesia euphorbiana* larvae on *E. lucida*.
June 13-14	Pecora to Pisa to collect *Dasineura* galls.
June 28-30	Clement to Puglia to collect *Apion* on yellow starthistle.
July 12-13	Cristofaro and Stazi to Pisa to collect *Dicranocephalus* and to work on *Dasineura* plots.
July 16-18	Clement and Mimmocchi to Puglia to collect biocontrol agents on *Centaurea* spp.
July 28-August 7	Clement to Greece to confere with Dr. Sobhian and to survey yellow starthistle in northern and central Greece.
August 18-September 15	Dunn to Canada to attend the VI International Symposium on the Biological Control of Weeds and to San Francisco and Washington to confere with colleagues and supervisors.
September 26-30	Dunn to Paris to participate to the Paris Laboratory Dedication

October 6-12	Pecora to Paris to present a paper at the Meeting of the European Working Group for the biological control of Weeds.
October 16-18	Dunn and Campobasso to Sila to collect Cheilosia.
October 19-31	Pecora and Cristofaro to Rumania and Austria to collect Chamaesphecia larvae on E. virgata.

Insect Shipments from the Rome Laboratory

Host Weed(s)	Location	Stage Date	No. Shipping Method	Receiving Location
Centaurea diffusa				
Sphenoptera jugoslavica	Saloniki Greece	500 larvae 5/22/84	Airfreight	Albany, CA.
Pterolonche inspersa	Rome Italy	780 eggs 8/22/84	Airfreight	Albany, CA.
Pterolonche inspersa	Rome Italy	550 eggs 9/4/84	Airfreight	Albany, CA.
Euphorbia esula				
Uromyces scutellatus	S.Rossore (Pisa),I	5/22/84	APO	Frederick, MD.
Bayeria capitigena	S.Rossore (Pisa),I	1500 eggs 5/23/84	Airfreight	Albany, CA.
Bayeria capitigena	S.Rossore (Pisa),I	2500 eggs 5/29/84	Airfreight	Albany, CA.
Bayeria capitigena	S.Rossore (Pisa),I	120 galls 6/4/84	Airfreight	Albany, CA.
Bayeria capitigena	S.Rossore (Pisa),I	210 galls 6/18/84	Airfreight	Albany, CA.
Aphthona flava	S.Rossore (Pisa),I	100 adults 6/26/84	Aifreight	Albany, CA.
Euphorbia lucida				
Euphorbia cyparissias				
Lobesia euphorbiana	Bosco Mesola Italy	455 larvae 6/25/84	Airfreight	Regina, Canada
Carduus nutans				
Pathogen smut	Monte Scalambra	8/14/84	APO	Frederick, MD.
Cirsium arvense				
Urophora cardui	Wien Austria	4000 galls 4/17/84	Airfreight	Albany, CA.

VISITORS (Rome Lab.)

Dr. Robert E. Perdue, USDA, ARC, Beltsville, MD.

Dr. Michael Julien, CSIRO, Brisbane, Australia

Dr. Sara Rosenthal, USDA, ARS, WR, Albany, California

Dr. Frank G. Zalony, IPM Implementation Group, Univ. of Calif., Davis, CA.

Dr. William Bruckart, USDA, Plant Disease Lab., Frederick, MD.

Mr. Boleslaus Kopacz, USDA, ARS, IA, Beltsville, MD.

Mr. Dominique Coutinot, USDA, ARS, EPL, Paris, France

Mr. Kim Chen, USDA, ARS, EPL, Paris, France

Dr. W.Calvin Welbourn, Acarology Lab., Ohio State Univ., Columbus, Ohio.

Mr. Don Maddox, USDA, ARS, WR., Albany, California.

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Dr. David G. Horn, Dept. of Entomology, Ohio State Univ., Columbus, Ohio.

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Dr. Arthus Nies, USDA, Deputy Adminitrator, Washington, D.C.

Dr. Angela Bazzoffia, University of Perugia, Perugia, Italy

Sister Mary Frances Traynor, Assisi, Italy

Sister Nancy Hutchinson, Assisi, Italy

Sister Karen Seronio, Assisi, Italy

Dr. David Briese, CSIRO, Canberra, Australia

Partial List of Recipients of this Report

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